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Application of Growth Regulators and Saprophytic Fungi *Trichoderma viride* Pers ex Fr. to Improve the Sanitary Condition of the Soil under Pepper Crops



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Abstract

The article presents studies of the effect of the microbiological preparation "Trichodermine", bio-organic fertilizers and growth regulators based on humic acids "Nagro", "Stimix", "BioLife" and mineral fertilizers (NPK) on the biological activity of the soil, the degree of disease caused by *Alternaria capsici-annui* Savul & Sandu and the yield of pepper in the field. It was found that the differences in the indicator "Biological Activity of the Soil" depend on the number of microorganisms, the composition (species diversity) and the percentage of the main Micromycetes. Organic products help to optimize soil health and reduce the total content of fungal colonies, and in the composition of Micromycetes, the fungus *Trichoderma* sp. is more common. When mineral fertilizers are applied, the number of fungal pathogens and toxin producers increases. A higher total yield was obtained in the variants with biological treatment. The yield increase is from 9% to 29%.

Keywords: vegetable production, biological activity of the soil, resistance to diseases

Introduction

The development of fungal diseases in vegetable field crops is one of the limiting factors of yield. Black leaf spots (*Alternaria capsici-annui* Savul & Sandu) - one of the most important economic diseases on pepper. According to some authors (Chulkina, 1995; Bakker et al., 2014), the intensity of the disease depends on the amount of soil-seed infection and ecological and technological conditions of cultivation. The concept of biological protection of plants is determined by the amount of organic matter in the soil, the introduction of organic fertilizers and the use of biological agents (Rudakov, 2006).

Growth regulators and organic fertilizers based on natural materials have received considerable attention from both the scientific community and commercial enterprises, especially in the last two and a half decades (Apone et al., 2010; Craigie, 2011; Brown & Saa, 2015; Du Jardin, 2015; Yakhin et al., 2017). Biostimulants offer a potentially novel approach to regulating / modifying physiological processes in plants to stimulate growth, relieve stress and increase yields (Belakbir et al., 1998; Bhattacharyya & Jha, 2012; Ikrina & Kolbin, 2004; Linser et al., 2006; Mora et al., 2010). The effect of organic fertilizers on the intensity of vegetable crop diseases has been studied by a number of researchers (Colla et al., 2015; Di Marco & Osti, 2009; Dixon & Walsh, 2004; Fuentes-Ramirez & Caballero-Mellado, 2006;

Hernandez et al., 2014; Hernandez-Herrera et al., 2015). It was found that the use of organic fertilizers on greenhouse tomatoes changes the nutrition balance and affects the phytosanitary status of this crop (Georgieva, 2013).

The aim of this work is to study the effect of new growth regulators on the following indicators in the production of pepper: the total microbiological activity and structural composition of soil Micromycetes, the index of damage by *Alternaria* black spots, the total yield and percentage of standard products.

Materials and Methods

Experimental work was carried out in 2009-2011 on the territory of Maritsa Vegetable Crops Research Institute on alluvial-meadow soil with pepper variety "Kurtovska kapia". Soil characteristics: humus content-2.1% (Turin), mineral nitrogen($\text{NH}_4 + \text{NO}_3\text{-N}$), determined by the distillation method-1.8 mg/100 g soil, mobile P_2O_5 -16.1 mg/100 g and mobile K_2O – 18.2 mg/100 g soil (Egner-Rhyme), soil reaction $\text{Ph}/\text{H}_2\text{O}/-$ 6.9 (potentiometric). The experiment was carried out using the block method in four replications (100 replicated plants), with a test area of 12.8 m². The strategic direction of the experiment included the study of the influence of the *Trichoderma viride* Pers, the bio-fertilizers „Nagro”, „Stimix”, „BioLife” on the overall soil biogenicity, the composition and structure of soil Micromycetes and the development of black spots (*Alternaria capsici-annui* Savul & Sandu).

For this purpose, the pepper seeds were treated with "Nagro" and "Stimix" biofertilizers at a concentration of 1:100 for 4 h followed by treatment of plants with the same bio-fertilizers at a concentration of 1:100 during the flowering phase and after the first harvest. „BioLife” was introduced into the rhizosphere as a liquid solution at a concentration of 500ml/ 200L H₂O at 5L/m² immediately after planting, during the flowering period. The biological product "Trichodermine" (*Trichoderma viride* Pers ex Fr.) was introduced into the soil at a dose of 40 kg/Ha. Experimental variants were performed on a standard and twice-reduced mineral background. The standard mineral background contained N16P20K22: ammonium nitrate - 500 kg/Ha, super phosphate - 450 kg/Ha, potassium sulfate - 450 kg/Ha. Phosphorous and potassium fertilizers were applied during the autumn tillage, nitrogen fertilizers - during the growing season. The reduced mineral background contained N8P10K11, respectively.

Indicators: Total% Yield (kg/Ha), Attack Index of black spots on leaves affected by *Alternaria*, Percentage of Affected fruits (%), Total Biological Activity of the Soil (the number of CFU per 1 g of dry soil, the number of fungal colonies in 1 g of dry soil). The frequency of *Alternaria capsici-annui* attacks was calculated on a five-digit scale (Gannibal et al., 2011): 0 - no symptoms, 1- single spots, 2- up to 25%, 3- 26-50%, 4- more than 50 % of the leaf surface is covered with spots, the plant is strongly suppressed. The attack index was calculated using Mc. Kinnly formula, the effect of using organic fertilizers - according to the Abbott formula. During mass harvesting, the percentage of diseased fruits with signs of *Alternaria capsici-annui* was calculated (%). Microbiological analysis of soil samples was performed at the beginning, middle and end of the growing season - after harvesting. The biological activity of the soil was determined by standard microbiological methods of cultivation of microorganisms on elective nutrient media (Zvyagintsev, 1991).

Statistical processing of the obtained experimental data was performed using Excel (Microsoft Office 2002), Windows XP. The results of the study were analyzed using a standard statistical procedure and presented as average values from three replicas.

Characterization of bio-organic liquid fertilizers:

„Nagro” - 19.17% dry substance; 10.29 g/l organic substance; Ph-8.5; N(common)-0.77g/L; phosphorus (P₂O₅)-387 ml/L; potassium (K₂O)-4624 ml/L; C:N (6:1); micro elements Cu-0.51 ml/L, MgO-541 ml/L, Mo-669 ml/l, S-1.8%, Zn-6.0 mg/Kg, Fe-154ml/L, B-1.02 ml/l, humic acids-1.63 g/L, polyacids-8.67 g/L;

„Stimix” - free amino acids-15-30 g / l, humic acids-30-50 g/l, organic silicon, live microbial cultures, biologically active substances.

„BioLife” - is a combined highly concentrated bacterial preparation. It contains enzymes and non-pathogenic microorganisms, as well as an active starter culture that accelerates and enhances the development of microorganisms and stimulates the direct action of soil bacteria.

„Trichodermine” (*Trichoderma viride* Pers ex Fr., strain No 12) is a biological product with a titer of $2 \cdot 10^{10}$ c/g, obtained by solid-phase technology.

Results and Discussion

The results of our research confirm that the sanitary condition of the soil depends on the total effect of factors associated with the cultivation of agricultural crops. Microbiological analysis of soil samples taken from the pepper field at the beginning, middle and end of the growing season, shows that the biological activity of the soil in the experiment changes throughout the growing season (Table 1). The Total Biological Activity of the Soil in the experiment with pepper increases twice during the growing season. The increase in the biological activity of the soil in the control variant with mineral fertilizers (N16P20K22) during the flowering phase - the first harvest is 40%, at the end of the growing season – 26%. This shows that with standard pepper fertilization with a complex of mineral fertilizers (NPK), the mineralization process is most intense in the middle of the growing season, when the first harvest occurs. At the end of the growing season, the biological activity of the soil is still high, but with lower values than calculated in the middle of the growing season. The distribution of microbial colonies indicates an increase in the number of bacteria and Actinomycetes in the middle of the growing season, followed by an equalization of this indicator by the end of the period in almost all variants.

The number of fungal colonies is greatest at the beginning of the growing season and varies depending on the use of fertilizers and biologics (Table 1). The amount of imported fertilizers affects the biological activity of the soil. The introduction of a standard dose of mineral fertilizers (N16P20K22) in the control variant enhances the process of biological activity. The number of fungal colonies in 1 g of soil in the middle of the growing season is the highest in the variants of full mineral fertilizer-N16P20K22. Probably, the main factor regulating the number of microorganisms in the soil is the nitrogen content. It is well known that the study of the influence of the structural composition of the complex of Micromycetes in the soil is limited to four functional groups of fungi: saprophytes, parasites, antagonists, and fungi-producers of toxins. The variant with mineral fertilizers is dominated by fungi that produce toxins-*Penicillium* sp., *Aspergillus* sp. and *Fusarium* sp. (Table 2). The use of

“Trichodermine”, “Nagro”, “Stimix” and “BioLife” bioproducts leads to optimization of the sanitary state of the soil by reducing the content of fungal colonies in variants with bioproducts.

Table 1. The content of germs in soil samples during the years of the experiment.

Season/ Variant	Number of microorganisms in 1 g of dry soil								
	Bacteria, mn/g			Actinomycetes, mn/g			Micromycetes, thsd/g		
	1	2	3	1	2	3	1	2	3
Start of the Vegetation Period									
Total sample	1,17	1,11	1,14	0,09	0,14	0,12	31,65	45,00	38,33
The Middle of the Vegetation Period									
1 (N ₁₆ P ₂₀ K ₂₂)	2,43	1,99	2,21	0,07	0,14	0,11	52,50	17,00	34,75
2 (N ₈ P ₁₀ K ₁₁)	0,87	1,87	1,34	0,23	0,18	0,21	26,50	27,00	26,75
3 (N ₈ P ₁₀ K ₁₁) + “Trihodermine”	0,59	1,84	1,22	0,05	0,14	0,10	17,50	18,00	17,75
4 (N ₈ P ₁₀ K ₁₁) + “BioLife”	1,44	2,34	1,89	0,07	0,27	0,17	20,00	28,00	24,00
5 (N ₈ P ₁₀ K ₁₁) + “Nagro”	1,81	1,41	1,61	0,27	0,12	0,20	32,50	18,00	25,25
6 (N ₈ P ₁₀ K ₁₁) + “Stimix”	0,66	2,10	1,38	0,33	0,17	0,25	25,00	16,00	15,50
The End of the Vegetation Period									
1 (N ₁₆ P ₂₀ K ₂₂)	1,71	1,62	1,67	0,05	0,13	0,09	24,75	30,00	27,38
2 (N ₈ P ₁₀ K ₁₁)	0,63	1,20	0,92	0,04	0,20	0,12	11,75	36,00	23,88
3 (N ₈ P ₁₀ K ₁₁) + “Trihodermine”	0,52	0,51	0,52	0,18	0,10	0,14	20,00	27,00	23,50
4 (N ₈ P ₁₀ K ₁₁) + “BioLife”	2,03	2,07	2,05	0,08	0,16	0,12	42,50	24,00	33,25
5 (N ₈ P ₁₀ K ₁₁) Nagro	0,64	1,50	1,07	0,18	0,13	0,47	13,00	12,00	12,50
6 (N ₈ P ₁₀ K ₁₁) + “Stimix”	2,45	1,04	1,75	0,10	0,06	0,08	6,25	13,00	9,63

The content of soil fungi taken from the variants with "Trichodermine" and "BioLife" is significantly lower than in the control variants, and Trichoderma colonies are more common. The effect of bio-products is better expressed on a reduced mineral background. Therefore, the introduction of "BioLife" into the rhizosphere when planting on a twice-reduced background increases the biological activity of the soil by 51% by the middle and by 43% by the end of the growing season. The study of the composition of microorganisms in the soil shows that the number of bacteria in this variant doubles - from 1.14 million/g at the beginning to 1.89 million/g by the middle and to 2.05 million/g of dry soil by the end of the growing season. Accordingly, in the same variant, the growth of Actinomycetes ranges from 0.14 million/g to 0.27 million/g in dry soil (Table 1).

Table 2. The structural composition of Micromycetes in soil samples.

№	Variant	Fungi colonies in petri dishes, per sp.cent							
		<i>Alternaria</i> sp.	<i>Fusarium</i> sp.	<i>Trichoderma</i> sp.	<i>Penicillium</i> sp.	<i>Aspergillus</i> sp.	<i>Mucor</i> sp.	<i>Cladosporium</i> sp.	<i>Mycelia sterilia</i>
1	(N ₁₆ P ₂₀ K ₂₂)	17,65	5,89	0,00	5,89	17,65	43,00	5,89	10,00
2	(N ₈ P ₁₀ K ₁₁)	5,50	0,00	5,50	2,25	21,75	6,25	5,25	23,50
3	“Nagro”	3,75	5,25	5,50	8,50	22,25	26,75	7,25	10,75
4	“Stimix”	0,00	0,00	6,66	6,66	23,33	30,00	2,00	12,25
5	“Trichodermine”	0,00	0,00	7,25	0,00	12,25	10,00	0,00	20,50
6	“BioLife”	0,00	3,50	6,50	6,95	23,75	5,00	7,15	37,15

The content of Micromycetes in the soil is maximum at the beginning of the growing season, after which the number of fungi decreases both in the variant with mineral nutrition and in the variant with bioproducts. The content of micromycetes in the soil is maximum at the beginning of the growing season, after which there is a decrease in the number of fungi both in variants with mineral nutrition and in variants with bioproducts. The introduction of bio-products on a low mineral background of bio-products creates favorable conditions for the development of microorganisms of all major groups. This guarantees the stability of functional relationships between the main ecological and trophic groups of microorganisms. The positive effect of biofertilizers on the development of fungal diseases of vegetables has been proven by many researchers (Egoshin, 2008; Yakovlev, 2006).

Monitoring of the incidence during harvesting showed differences in the rate of damage from *Alternaria capsici-annui*, depending on the rate of application of mineral (NPK) fertilizers and the applied biological product. In variants with mineral fertilizers, the development of black spots of *Alternaria* is 30-32%, with "BioLife" - 23-29% (Table 3).

The standard rate of basic mineral fertilizers (N₁₆P₂₀K₂₂) is most effective for growing plants treated with “Nargo” and “Stimix” biologics. In variants with treatment of seeds with "Nagro" solution, the incidence reduction is 45% against the background (N₈P₁₀K₁₁) and 55% against the background (N₁₆P₂₀K₂₂). Seed treatment with “Stimix” solution reduces the development of black spots in pepper by 6% against the background (N₈P₁₀K₁₁) and by 47% against the background (N₁₆P₂₀K₂₂). Compared with the control variants, the effect of BioLife biofertilizer ranges from 11% on a reduced to 28% on a standard mineral background. A single treatment of the soil with the "Trichodermine" bioproduct gave good results for both fertilizer standards - standard (N₁₆P₂₀K₂₂) and twice reduced (N₈P₁₀K₁₁). The greatest effectiveness against black spots of *Alternaria* in seeding (56%) was registered when using the biological product "Trichodermine".

Table 3. *The influence of the organo-mineral system of fertilizers and bioproducts on the development of Alternaria black spots and the yield of sweet pepper.*

Variant	Alternaria black spots, per cent	Effect, per cent	Yield, kg/Ha	Effect, per cent	Standard production, per cent
Control	32,10	-	37730,01	-	66,76
(N ₁₆ P ₂₀ K ₂₂)	29,55	7,94	41490,20	9,79	87,82
(N ₁₆ P ₂₀ K ₂₂) + Nagro	14,5	54,82	46850,71	19,48	98,45
(N ₈ P ₁₀ K ₁₁) + Nagro	17,55	45,33	50570,14	25,39	98,96
(N ₁₆ P ₂₀ K ₂₂) + Stimix	20,55	35,98	48520,38	22,24	97,00
(N ₈ P ₁₀ K ₁₁) + Stimix	17,05	46,88	49870,57	24,35	92,54
(N ₁₆ P ₂₀ K ₂₂) + Trichodermine	14,05	56,23	49530,96	23,84	93,21
(N ₈ P ₁₀ K ₁₁) + Trichodermine	13,97	56,48	52110,15	27,60	97,72
(N ₁₆ P ₂₀ K ₂₂) + BioLife	28,65	10,75	53580,73	29,59	89,21
(N ₈ P ₁₀ K ₁₁) + BioLife	23,25	27,57	43880,89	14,033	94,81

Our results confirm that the use of microbial products in vegetable crops promotes growth, yield, and product quality. It was found that when growing field pepper, productivity by treating seeds with growth regulators and applying "Trichodermine" mineral fertilizer to the soil can be reduced by half without the risk of crop loss from *Alternaria capsici-annui*. As can be seen from table 3, the yield of pepper varieties "Kurtovskaya kapiya" in the version with a standard dose of mineral fertilizers was 41490 kg/Ha and 37730 kg/Ha on a reduced mineral background. Compared to a reduced mineral background, the yield increase on a standard mineral background is 10%. Treatment of pepper seeds with humic acids "Nagro" and "Stimix", treatment of the plant's rhizosphere with biological fertilizer "BioLife" and introduction of the biological product "Trichodermine" into the soil before planting enhance the effect of using complex mineral fertilizers. In the variants with "Nagro" and "Stimex", the yield increase is in the range of 19 – 24%, depending on the amount of mineral fertilizers in the experiment. On a reduced background of mineral fertilizers (N₈P₁₀K₁₁), the effect of these drugs is higher than on the background created by the introduction of a full dose of mineral fertilizers (N₁₆P₂₀K₂₂). The use of Trichodermin bio-product on a mineral background (NPK) increases the yield in field production of pepper by 27%. A significant increase in yield by 29% was observed with the introduction of "BioLife" on a mineral background (N₁₆P₂₀K₂₂). This is probably due to the fact that BioLife helps the nitrification process and accelerates the absorption of mineral nitrogen by bacteria. An integrated system of organic and mineral fertilizers improves the quality of the products. The percentage of standard production is the lowest in the reduced mineral variant (N₈P₁₀K₁₁) - 67%. In the variant using (N₁₆P₂₀K₂₂), it is 88 percent. For comparison, 89-98% of standard products were obtained in variants using organic products "Nagro", "Stimix", "Trichodermine" and "BioLife".

Conclusion

The amount of imported mineral fertilizers affects the biological activity of the soil. The introduction of a standard dose of mineral fertilizers (N16P20K22) enhances the process of biological activity.

The use of "Trichodermine", "Stimix" and "BioLife" bio-products optimizes soil health by reducing the number of fungal colonies in the variants with bio-products.

The effect of bio-products is better expressed on a reduced mineral background.

The introduction of bio-products on a low mineral background creates favorable conditions for the development of microorganisms of all major groups. This ensures the stability of functional relationships between the main ecological and trophic groups of microorganisms.

The effectiveness of "Trichodermine" against black spots of *Alternaria* in pepper sowing is 56%. In variants with the use of "Nagro" for seed treatment, the incidence reduction is 45% against the background of N8P10K11 and 55% against the background of N16P20K22.

The use of an integrated system of fertilizer application (organic, mineral fertilizers and biological product "Trichodermine") increases the yield and yield percentage of standard pepper in the field. The increase in yield is 19-24% with the use of biofertilizers "Nagro" and "Stimix", 23% - "Trichodermine" and 29% - "BioLife".

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Some Results of Stationary Sample Plot for Intensive Monitoring of Forest Ecosystems in Vitinya Area

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Abstract

Results are presented for the chemical composition of the forest litterfall and lysimetric waters of Cambisols from the intensive monitoring stationary sample plot of the forest ecosystems. Litterfall (litter biomass fraction) had fallen on the soil surface with acid reaction, which in the period 2011 - 2018 varied from 5.1 to 5.4. The established contents of calcium (6.9 mg.g^{-1}), magnesium (0.52 mg.g^{-1}), potassium (3.58 mg.g^{-1}), nitrogen (7.28 mg.g^{-1}) and phosphorus (0.55 mg.g^{-1}) were low and those of manganese ($1754 \text{ }\mu\text{g.g}^{-1}$), iron ($185 \text{ }\mu\text{g.g}^{-1}$), copper ($11 \text{ }\mu\text{g.g}^{-1}$) and zinc ($36 \text{ }\mu\text{g.g}^{-1}$) were high. The lysimetric waters in the surface horizon were very strongly acidic – $\text{pH}_{\text{H}_2\text{O}} = 4.6$. The amounts of all the tested parameters in them were very low – dissolved organic matter (8.0 mg.dm^3), potassium (2.6 mg.dm^3), calcium (5.7 mg.dm^3) and others. Only the content of manganese was increased, which in some years exceeded the maximum value pointed in the literature for the European forests. It is assumed that degradation processes take place in the mineral part of the soil which lead to its impoverishment of macro and microelements.

Key words: litterfall, lysimetric waters, common beech, Cambisols

Introduction

In the forest ecosystems, nutrients make internal circulations along the 'entrance and exit' paths in them. The entrance is implemented through the litterfall, wet and dry depositions, stemflow, and the exit - through cuttings, lysimetric waters, etc. (Smidt et al., 2012). Their behavior inside the soil depending on its reaction, adsorption surfaces, and others. The results of the impact of anthropogenic activity are evaluated by the displacement of the basic cations from the litter (L) and the soil, the occurrence of calcium and magnesium deficiency in the leaves of tree species, and others (Kiikkilä, 2003).

The main sources of nutrients for the soil are litterfall and weathering processes. Litterfall becomes very important when in the soil-plant system the biological cycle dominates over the geochemical one. According to Legout et al. (2014) in rich soils, nutrient amounts are result of weathering processes, i.e. from the dominance of the geochemical circle. However, the intensity of weathering is determined by the climatic conditions, and for the mountainous territories in the country they are most often characterized as "harsh".

Litterfall accumulates on the soil surface where it forms the litter. It is considered to be the most dynamic and rapid flow of nutrients to soil in forest ecosystems. Litterfall is an important source not only of nutrients but also of organic carbon for the soil (Sayer, 2006). It has been considered by some authors as a key component in assessing soil organic matter retention in global warming conditions (Liski et al., 2005; Hansen et al., 2009).

The aim of the study was to evaluate the amounts of nutrients in the litterfall and lysimetric waters in the intensive monitoring stationary sample plot in Vitinya area, obtained for 2018 and their dynamics in the period 2011 - 2018.

Materials and Methods

Vitinya stationary sample plot is located in the western part of the Central Balkan Mountains, on the territory of Vitinya State Forestry. It was selected as representative of the natural beech (*Fagus sylvatica* L.) forests in the country (Kolarov et al., 2002). The altitude is 950 m. It is located in the Mountain Climate Region, Middle Mountain Subarea (Koleva - Lizama, 2002). The soil is Dystric Cambisols, formed on gneiss.

Litterfall was collected in 5 pcs. catchers, each with an area of 1 m². It was collected every two weeks, separating in 3 major fractions according to the Methodological Monitoring Manual, Part 15 (ICP Forests, 2006). The fractions were as follows: litter (L), wood fraction (WF), which includes bark, branches, twigs and others with area exceeding 5 x 5 mm or with diameter more than 2 mm, and fruits and seeds (FS).

Eight sites were created to collect lysimetric waters, from which mixed samples were made. The lysimeters were placed at such depth so they can collect water from the surface and from the metamorphic horizons. Samples were collected every two weeks.

Sampling and laboratory analyzes of the observed parameters were carried out by the Executive Agency for the Environment towards Ministry of Environment and Waters in connection with the implementation of the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) – Level II, within the framework of the Convention on Long-range Transboundary Air Pollution of the United Nations Economic Commission for Europe.

Results and Discussion

The total biomass of litterfall was observed for a longer period (2009 - 2018) than other indicators, and it varied significantly (Fig. 1). The most stable was the biomass of the L fraction. The other 2 fractions - WF and FS were characterized by large variation due to the different amounts of bark, twigs, reproductive organs, and others that fall into the catchers. Fraction WF may even be absent. Also important was the fruiting of the common beech, which is not annual.

Table 1. Contents of macro- and microelements in litterfall from Vitinya Stationary sample plot, 2018.

Catcher №	Fraction	pH _{H2O}	N	S	P	Ca	Mg	K	org.C	Zn	Mn	Fe	Cu	Pb	Cd
			mg/g						g/100g	µg/g					ng/g
1	Litter	5.36	7.95	0.99	0.55	4.29	0.69	4.91	47.82	26.76	2867.8	180.78	7.24	0.79	198.22
2		5.48	5.63	0.86	0.31	9.06	0.28	1.15	47.87	44.20	911.7	135.53	13.75	2.09	392.91
3		5.60	8.25	0.75	0.94		0.59	6.06	48.20	12.02	714.2	124.27	9.97	0.59	195.40
4		5.37	7.50	0.85	0.52	4.47	0.73	4.55	48.11	27.28	3138.7	214.73	6.13	0.59	197.00
5		5.38	7.09	0.94	0.46	9.80	0.36	1.27	48.32	72.38	1142.4	270.04	16.16	9.11	489.69
mean		5.43	7.28	0.88	0.55	6.90	0.52	3.58	48.06	36.52	1754.95	185.07	10.64	2.63	294.64
st.dev		0.10	1.02	0.09	0.23	2.92	0.20	2.24	0.21	23.05	1153.55	56.72	4.25	3.67	138.12
1	Wood Fraction	5.55	10.06	0.85	1.10		0.54	6.02	47.20	13.09	919.0	94.24	9.70	0.39	193.92
2		5.35	8.66	0.89	0.59	4.82	0.76	4.71	47.17	29.04	2934.2	285.81	8.86	0.98	196.89
3		5.3	6.13	0.87	0.51	4.79	0.29	1.17	46.92	41.84	811.6	151.94	11.53	10.56	387.42
4		5.43	10.07	0.79	1.04		0.74	6.28	47.49	15.70	1004.3	101.54	10.69	0.39	192.68
5		5.33	7.92	1.07	0.38	4.96	0.79	4.82	41.98	31.96	3008.0	197.92	7.86	0.70	199.11
mean		5.39	8.57	0.98	0.72	4.85	0.62	4.59	46.15	26.38	1735.38	166.29	9.72	2.60	234.00
st.dev		0.10	1.64	0.10	0.32	0.09	0.21	2.04	2.34	11.91	1130.37	78.85	1.44	4.45	85.80
1	Fruits and Seeds	5.25	7.26	0.94	0.34	8.26	0.32	1.42	47.88	49.54	937.0	178.49	15.99	8.44	490.46
2		5.37	5.28	0.75	0.60		0.40	6.51	45.56	12.62	600.60	91.88	7.51	0.29	192.62
3		5.23	11.07	0.96	0.58	4.44	0.91	4.45	46.35	30.09	2582.01	221.34	10.89	0.89	197.98
4		5.31	5.98	0.92	0.29	6.97	0.36	1.52	46.74	41.07	833.75	169.55	12.01	5.23	387.48
5		5.29	7.13	0.79	0.71		0.44	7.20	46.49	9.18	655.95	69.66	8.50	0.49	195.40
mean		5.02	7.60	0.92	0.81	18.62	1.31	4.83	53.47	34.72	885	413	7.11	2.72	236.52
st.dev		0.08	0.82	0.04	0.02	2.10	0.14	0.40	0.70	6.74	252	399	0.74	0.50	46.93

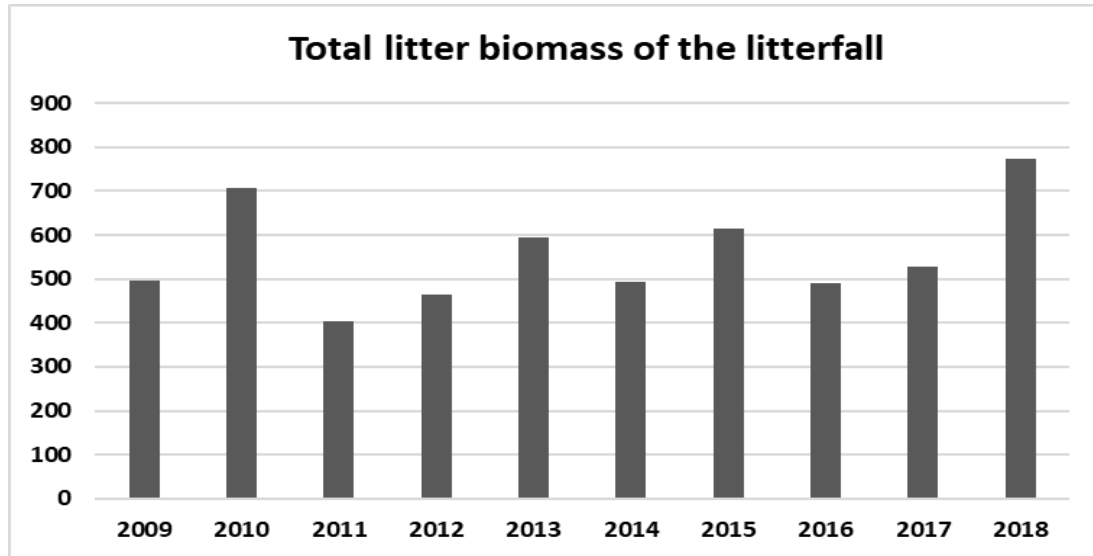
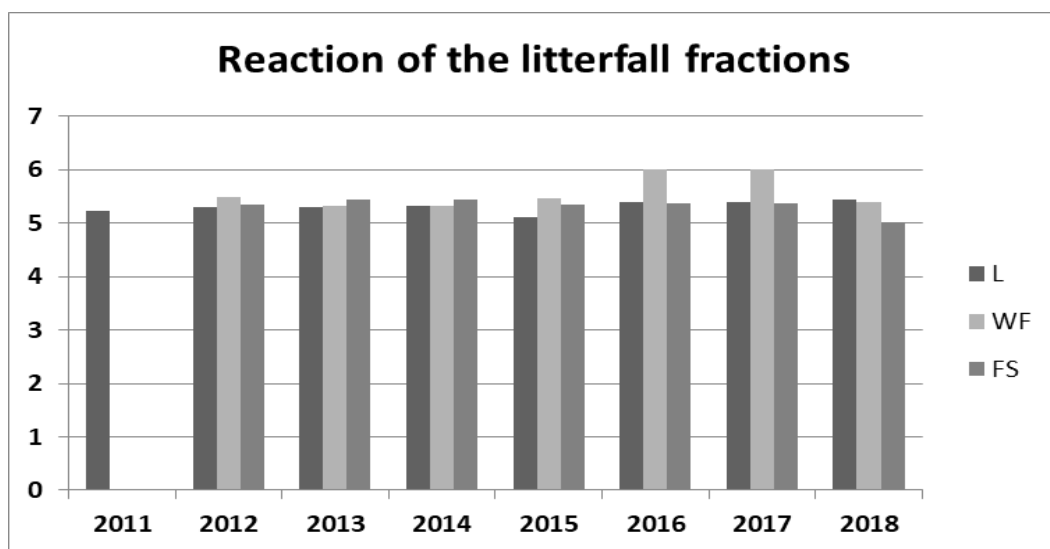


Fig. 1. Total litter biomass of the litterfall for the period 2009-2018 (g/m²)

The maximum leaf fall was in the range of 1 month – October, but there were also periods of smooth flow of this process – for 2 months, mainly October and November. Fraction WF was forming more active at the beginning of the vegetation season – April, and in its end – October. For the FS fraction, this period was in September–October.

Table 1 presents the litterfall reaction and the content of macro- and microelements by fractions. The reaction of litterfall was in the acid spectrum. For the L fraction, values close to those of previous years were obtained in 2018 (Fig. 2). An exception was found in 2015 as the results differ sharply. The lowest pH value was then obtained, with a statistically significant difference compared to 2013 ($p = 0.014$) and 2014 ($p = 0.014$).

Significant differences in the reaction of fraction WF - in 2016 and 2017 were established. They were not confirmed in time. It can be assumed that they were with incidental origin.

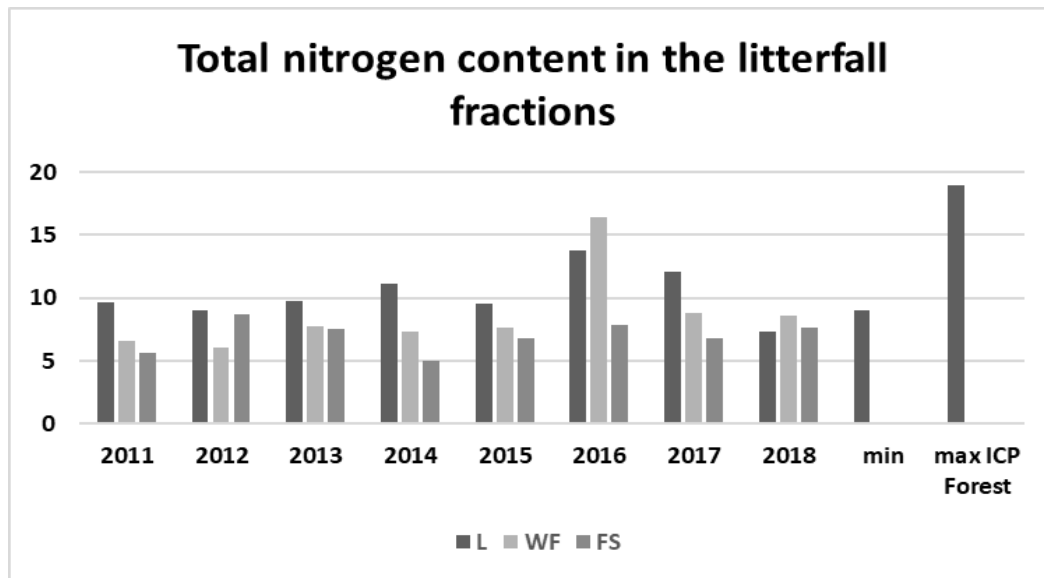


L –Litter. WF-Wood fraction. FS– Fruits and seeds

Fig. 2. Reaction of the litterfall fractions for the period of 2011-2018.

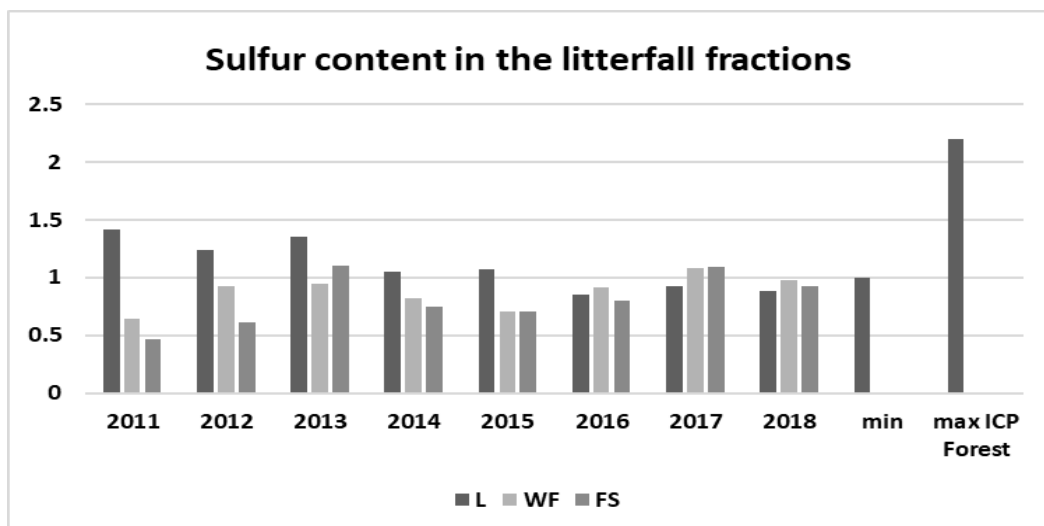
The nitrogen content of litterfall for the period 2011 – 2015 was low, close to the minimum value specified in the criteria of the ICP Forests Guide (2016). Higher values were reported in 2016 and in particular in 2017 (Fig. 3). In 2018, the tendency for lower nitrogen content in litterfall was reversed.

Regarding the amount of phosphorus, it can be said that its return to the soil through the litterfall was slightly higher than the minimum value specified in ICP Forests Guide Part 15 (2016), which was satisfactory.



L –Litter. WF-Wood fraction. FS– Fruits and seeds

Fig. 3. Total nitrogen content (mg.g⁻¹) in litterfall fractions for the period of 2011 -2018 and ICP Forest criteria.



L –Litter. WF-Wood fraction. FS– Fruits and seeds

Fig. 4. Sulfur content (mg.g⁻¹) in litterfall fractions for the period of 2011-2018 and ICP Forest criteria

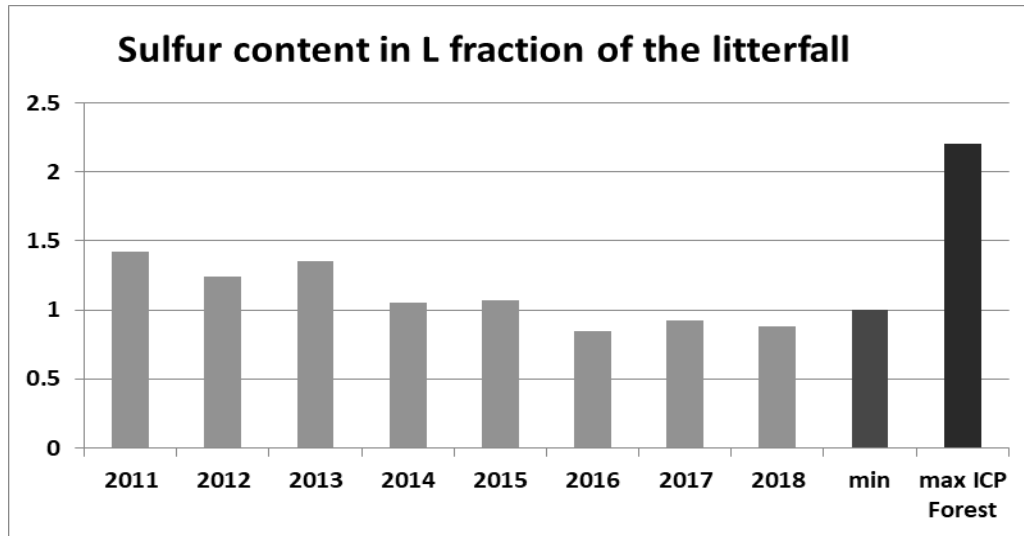


Fig. 5. Sulfur content (mg.g^{-1}) in „Litter” fraction in litterfall for the period of 2011-2018 and ICP Forest criteria.

The sulfur content continued to show a decreasing trend. In previous years, even lower concentrations were measured than those obtained in 2018 (Fig. 4). All results were below the minimum pointed out in ICP Forests Guide Part 15 (2016). This was most relevant for the L fraction (Fig. 5).

The calcium and magnesium macronutrients in the L fraction were low throughout the observation period. These of potassium also, as they varied around the minimum value. Given that this fraction (L) was the main source of organic matter for the soil, it could be assessed that the input quantities of basic elements were low.

The results showed a lasting tendency for an increased amount of microelements – manganese, iron, copper and zinc. As in previous years, they were high. This is most pronounced for the more mobile elements especially for manganese (Fig. 6) and copper which were observed from 2009 to 2018 (Fig. 7), but for the manganese there was a lack of data for 2017 and 2018.

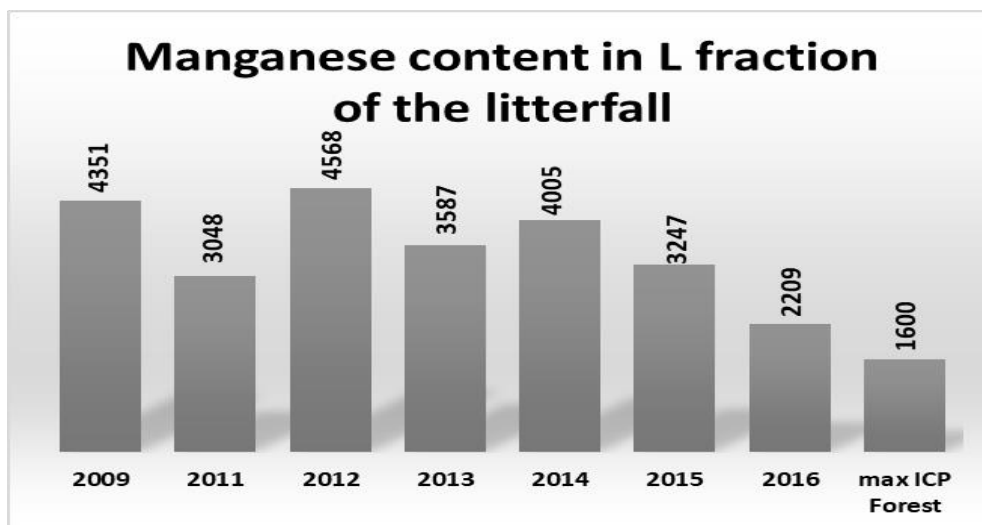


Fig. 6. Manganese content ($\mu\text{g.g}^{-1}$) in „Litter” fraction in litterfall for the period of 2009-2018 and ICP Forest criteria

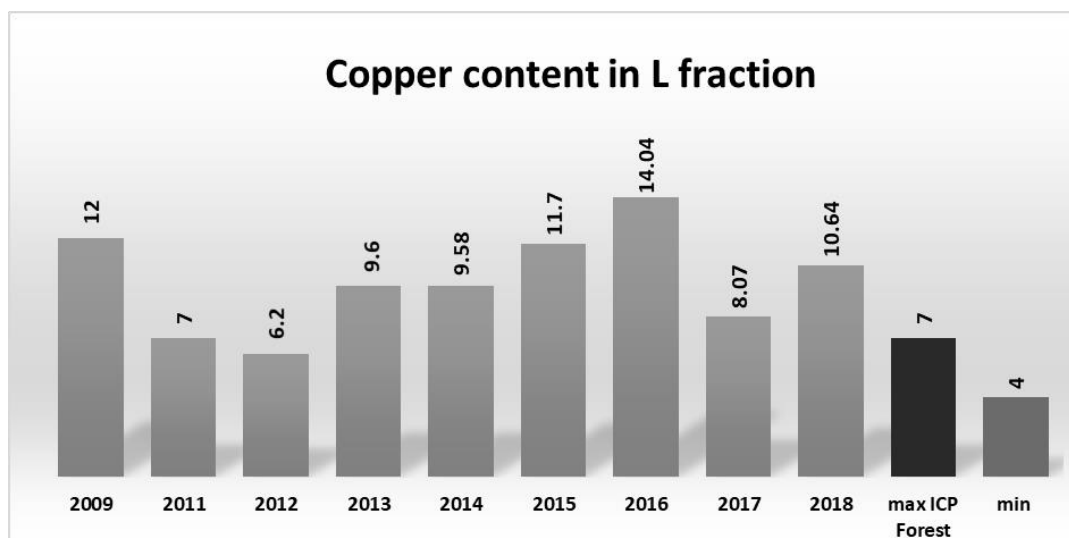


Fig. 7. Copper content ($\mu\text{g.g}^{-1}$) in „Litter” fraction of litterfall for the period of 2009-2018 and ICP Forests criteria.

Lead and cadmium values in 2018 varied more than in previous years. They were not assessed as high, but for both elements in the L fraction there was one high concentration obtained for at least 1 catcher. For now, it is appropriate to observe this fact because it also concerns the content of iron and zinc, which allows contamination of the samples.

Most of the studied elements were unevenly distributed in the different fractions. Usually, the L fraction accumulated higher amounts of microelements. The results of the FS fraction were strong in the direction of high accumulation of calcium, potassium, phosphorus and iron.

High amounts of microelements and low macroelements were explained by the very high soil acidity. In it occurred processes of mobilization of manganese, copper, zinc and other microelements and leaching of basic cations. The sum of the basic elements entering the soil with the litterfall is very low. It is $59 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Malinova, 2014). For comparison with the other 2 stationaries for intensive monitoring, this amount was $139 \text{ kg ha}^{-1} \text{ yr}^{-1}$ in Hungarian oak (*Quercus frainetto* Ten.) stand (Staro Oryahovo stationary sample plot) and from European spruce (*Picea abies* (L.) Karst) from - $95 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Yundola stationary sample plot). The difference with common beech came mainly from calcium and potassium, which were low in beech litterfall.

The measured reaction of lysimetric waters is an indicator of the presence of free carbonic acid in the soil, as well as of organic acids of biogenic origin, which are the result of the dominant influence of acidic products in the soil formation process. It varies slightly over time (Fig. 8).

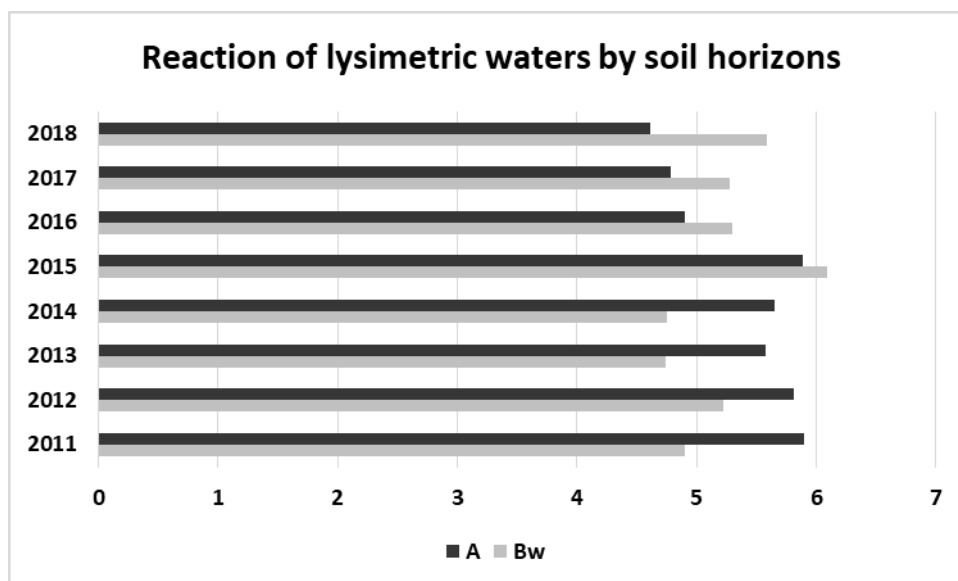


Fig. 8. Average pH values of lysimetric waters for the period 2011-2018

The alkalinity was low, which means that the neutralizing ability of the lysimetric waters was too low (Table 2). In some years, higher values were obtained - up to 265 $\mu\text{mol/l}$, which is also not high compared to the indicated minimum and maximum limits in the ICP Forests Guide. The conductivity of the solution was very low. It was dominated by chlorine ions, sulfates, nitrates, phosphates, ammonium ions. Base cations were also in low amounts.

Table 2. Active reaction, electrical conductivity and ionic composition of lysimetric waters, 2018.

Horizons	pH _{H₂O}	Alkalinity	Electrical conductivity	Dissolved organic matter	K	Ca	Mg	N-NO ₃	S-SO ₄	N-NH ₄
		$\mu\text{mol/l}$	$\mu\text{S/cm}$	mg/dm^3						
A	4.61	32	64.35	7.26	2.52	5.50	0.71	0.07	6.24	0.03
Bw	5.48	32	72.43	5.72	1.84	4.62	1.05	0.16	7.20	0.03

Table 2. (Continued).

Horizons	Cl	N (total)	Na	Fe	Mn	Zn	Cu	Pb	Cd
	mg/dm^3			$\mu\text{g/dm}^3$					
A	4.20	0.71	1.65	421.37	288.00	64.00	13.00	1.00	0.03
Bw	4.00	0.59	2.12	109.60	544.75	29.75	2.00	1.00	0.03

The manganese content was high (319 $\mu\text{g} / \text{dm}^3$ - 544 $\mu\text{g} / \text{dm}^3$) and in some cases also and iron. Manganese reached 1890 $\mu\text{g} / \text{dm}^3$ at an upper threshold of 1900 $\mu\text{g} / \text{dm}^3$ (ICP Forests Guidelines Part 11, 2016).

Conclusion

In Cambisols from the intensive monitoring stationary sample plot in the Vitinya area, there is a clear tendency for the litterfall chemical composition. Low amounts of macroelements nitrogen, phosphorus, sulfur, as well as basic elements and high amounts of microelements, mainly manganese and zinc, were returning to the soil. At the exit of the ecosystem - low amounts of water-soluble forms of the investigated macroelements, microelements, and salts were exported through lysimetric waters. It is important that forestry practices focus on limiting the export of dead biomass from the stands in order to recover the elements extracted from it through the root system of the plants.

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Plant Uptake of Radioactive Elements from Soils Contaminated by Uranium Mining Industry in Buhovo, Bulgaria

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Abstract

The aim of the present study was to determine the degree of accumulation in certain agricultural plants of uranium from soil collected from the bowl of Buhovo tailing pond near former uranium processing plant "Metallurg" in Bulgaria. The measured specific activities of uranium isotopes in soil were: ^{238}U - 2489 Bq/kg, ^{235}U - 117 Bq/kg and ^{234}U - 2107 Bq/kg.

Vessel vegetation trials were carried out with three varieties of barley (F-173, F-210, „Veslets”), triticale, chard, sorrel, arugula and maize on the collected soil. The highest soil to plant transfer coefficients were determined in leafy greens (^{238}U : $2,53 \cdot 10^{-3}$ - $5,97 \cdot 10^{-3}$; ^{235}U : $2,56 \cdot 10^{-3}$ - $6,07 \cdot 10^{-3}$; ^{234}U : $2,96 \cdot 10^{-3}$ - $6,06 \cdot 10^{-3}$), lower in cereals (^{238}U : $1,32 \cdot 10^{-3}$ - $1,99 \cdot 10^{-3}$; ^{235}U : $1,50 \cdot 10^{-3}$ - $2,09 \cdot 10^{-3}$; ^{234}U : $1,57 \cdot 10^{-3}$ - $2,49 \cdot 10^{-3}$) and the lowest in maize (^{238}U : $0,50 \cdot 10^{-3}$; ^{235}U : $0,56 \cdot 10^{-3}$; ^{234}U : $0,59 \cdot 10^{-3}$).

For leafy plants, the transfer factors for arugula and chard were up to three times higher than those for sorrel. Comparing different barley varieties, up to 50% lower accumulation was found in barley F173 than in other varieties tested.

Keywords: ^{234}U , ^{235}U , ^{238}U , uranium plant uptake

Introduction

Uranium mining and processing may cause a number of environmental problems. Common consequences are air, water and soil pollution due to physical and chemical weathering of extracted rocks as a result of the classical underground method of mining, or groundwater pollution, which by infiltration contaminates water and soils as result of so the called *in-situ* leaching method. In both cases, pollution occurs during mining, but may continue long after exploitation has ceased (Ettenhuber, Roehnsch, 1993).

Uranium mining in Bulgaria is one of the oldest in the world and dates back to 1946. 47 uranium deposits were found on the territory of the country, and most of them (over 30) were in operation.

Despite the measures undertaken so far on the elimination of environmental consequences from uranium industry, a number of problems related to the implementation of technical liquidation, water management and treatment and reclamation of contaminated soils still remain unresolved (Vapirev et al., 1993).

Increased uranium content in surface soil layers may lead to accumulation of the radionuclide and its daughter products in agricultural plants and pose radiological hazard to human health.

The aim of the present study was to determine the degree of uranium accumulation in some agricultural plants grown on soils contaminated by uranium industry and, if necessary, to grow on such soils plants that absorb the lowest amounts of the element.

Materials and Methods

Buhovo tailing pond is situated at about 500 m from the uranium processing plant "Metallurg", 15 km northeast from Sofia city in Bulgaria. The area is located on the southern slopes of Stara Planina, with an average altitude of 600 m to 700 m. The main water intake is the Yaneshtitsa River, which flows into the Lesnovska River and from there into the Iskar River. Development of uranium industry caused strong anthropogenic change of the area (Banov, Hristov, 1996; Yordanova et al., 2011). Industrial buildings, roads, embankments, a tailing pond, derivation canals, waste disposal sites along the valley of the Yaneshtitsa River, etc. were built.

The main type of soils in the studied area is the leached cinnamon forest soils, Chromic Cambisols according to World Reference Base for Soil Resources (WRB). Mechanical composition of the soils is heavy sandy-clayey to slightly clayey. The silt in the surface horizon is 35-45%, and the clay - 54-60%. The content of organic matter is low, in addition, most of it is located in the humus-accumulative horizon, and in the lower horizons its percentage decreases sharply. In the humus horizon it is 1.5-2.5%, and in the clay horizon - 0.9-1.5%. Content of total nitrogen is 0.08-1.11%. The well-defined leaching process in soils has led to a relatively deep deposition of carbonates in the profile and to a slight increase in soil acidity (pH – 6-7). The sorption capacity of the leached cinnamon soils is high and differentiated by the depth of the profile depending on the mechanical composition. It varies in the range of 30-35 meq per 100g of soil. These soils are saturated with bases of which calcium is predominating.

Pot experiments were carried out with three varieties of barley (F-173, F-210 and "Veslets"), corn, triticale, spinach, chard (*Beta vulgaris subsp. Vulgaris*), sorrel (*Rumex acetosa*) and arugula (*Eruca vesicaria sativa*) on uranium contaminated soil collected from the cup of Buhovo tailing pond. The experiments were carried out in three kilograms pots in three replications. Nitrogen, phosphorus and potassium were introduced into the soil during the vegetation period.

Agrochemical characteristics of the soil were determined by procedures validated in „N. Poushkarov” Institute of Soil Science, Agrotechnologies and Plant Protection.

Plants (stems and leaves) were dried to an air dry state.

^{238}U , ^{234}U and ^{235}U were determined by validated laboratory procedure for radiochemical determination of uranium isotopes in soils, plants, water and other environmental objects (Pimpl et al. 1992; Naydenov et al., 2010). The measurements were performed for 1000 min using low background alpha spectrometer with a PIPS semiconductor detector. The calibration was performed with a certified reference material containing plutonium-239, americium-241 and curium-244 in a 1:1:1 ratio, manufactured by Amersham. The uncertainty of results was calculated at $k = 2$ for 95% confidence level. The

measurements are validated by annual participations in interlaboratory comparisons and proficiency test schemes.

To assess the degree of uranium accumulation in plants, the corresponding transfer factors (TF) were determined - the ratio of activity concentration in 1 kg plant (dry weight) to the activity concentration in 1 kg soil (dry weight) (Sheppard, 1980).

Results and Discussion

The main chemical characteristics of the soil used in the pot experiment are presented in Table 1.

Table 1. Agrochemical and physicochemical characteristics of the soil

pH			$\sum N$ NH ₄ + NO ₃		P ₂ O ₅	K ₂ O	Soil organic matter %
H ₂ O	KCl		mg/kg		mg/100 g		
7.4	6.8		6.6		9.3	6.4	
T _{8,2}	T _{CA}	T _A	H _{8,2}	Al	Ca	Mg	Base Saturation
meq/100 g							
79.05	-	-	0.0	0.0	79.0	0.5	100.00

Specific activity of of uranium isotopes, ^{238}U , ^{234}U and ^{235}U in soil were 2489, 2107, and 117 Bq/kg, respectively. Activity concentration of uranium-238 is 62 times higher than the average of the soils in Bulgaria - 40 Bq/kg (UNSCEAR, 2000) and at about 31 times higher than the activity measured in undisturbed soils of the area 70-90 Bq/kg (Yordanova et al., 2015) indicating the anthropogenic impact. The ratio $^{234}U/^{238}U$ is 0.85, probably due to the so-called α -recoil effect and preferential leaching of ^{234}U from damaged crystal lattice of the host mineral in the soil (Pekala et al., 2010; Suresh et al., 2013).

The data for the content of uranium isotopes in different plants (air-dry weight) are presented in Table 3 at up to 10% uncertainty at $k = 2$ for 95% confidence level.

Table 2. Specific activity of uranium isotopes in plants, Bq/kg

Sample	^{234}U	^{238}U	^{235}U	$^{234}U/^{238}U$
Corn	1.26	1.33	0.06	0.95
Barley (F-173)	3.33	3.27	0.18	1.02
Barley (Veslets)	5.20	4.97	0.24	1.05
Barley (F-210)	5.24	4.96	0.25	1.06
Triticale	4.30	4.24	0.20	1.01
Sorrel	6.24	6.29	0.30	0.99
Chard	12.78	13.54	0.64	0.94
Arugula	12.51	14.86	0.71	0.84

The lowest specific activity concentration of uranium isotopes was measured in corn, in leafy vegetables - chard and arugula, the activities were 10 times higher. In sorrel and two of

barley varieties (F-210 and Veslets) the specific activities were of the same order, 5-6 times higher than in the corn. The activities in barley variety F-173 were lower than the measured in other varieties of barley and triticale.

$^{234}\text{U}/^{238}\text{U}$ activity ratio in plants was around 1, indicating the identical uptake mechanism of uranium isotopes. However in arugula, where higher accumulation of two isotopes was observed, the ratio was lower than 1 and near to that determined in the soil.

Transfer factors (TF) of ^{238}U , ^{234}U and ^{235}U in plants are presented in Fig.1.

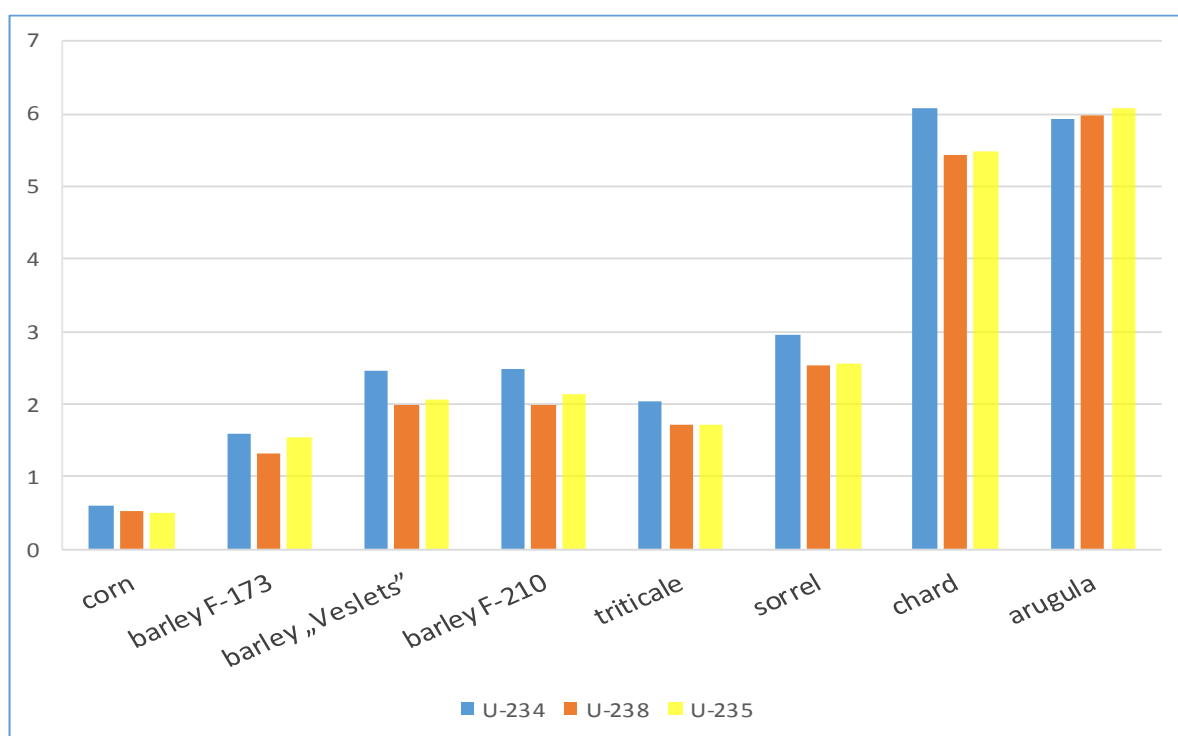


Fig. 1. Transfer factors of uranium isotopes in plants ($\text{TF} \cdot 10^{-3}$)

Highest transfer factors of U isotopes were determined in leafy vegetables, a trend observed by other authors too (Anke et al., 2009; Carvalho et al., 2016).

Transfer factors of ^{234}U , ^{235}U , ^{238}U in leafy vegetables and barley were comparable - $\text{Ft} = n \cdot 10^{-3}$, of corn was one order lower - $\text{Ft} = n \cdot 10^{-4}$. Differences of around 50% were observed between barley variety - F173 and varieties F-210 and Veslets.

As it can be seen from the results obtained, TFs of uranium isotopes determined in the plants were relatively low despite the relatively high activity concentrations measured in the soil.

For example Al-Masri et al. reported ^{238}U TFs of 0.8×10^{-2} and 1.2×10^{-2} in stems of barley and wheat respectfully, grown on soils with activity concentrations of ^{238}U between 11 and 33 Bq/kg (Al-Marshi et al., 2008). The TF values of ^{234}U and ^{238}U in cereals (wheat – leaves and stems) cited by Al-Hamarneh et al. were 0,28 и 0,29 respectfully, grown on soil with measured activities of two isotopes – 15,82 и 15,28 Bq/kg respectfully (Al-Hamarneh et al., 2016). Sasaki et al. reported TFs of 3.6×10^{-4} in spinach on soil from various districts of Japan (Sasaki et al., 2002).

In reference to soils contaminated by uranium mining industry, TFs between 0.011 and 0.023 were reported for lettuce (*Lactuca sativa* L.) grown on contaminated soils nearby abandoned mine in Cunha Baixa, Portugal (Neves et al., 2008).

In general TFs determined in the present study were lower or corresponding to the range 10^{-2} – 10^{-3} cited by IAEA as parametric values for the prediction of radionuclide transfer in temperate environments (IAEA, 1994).

As it is recognized uranium transfer coefficients vary widely depending on plant species, environmental conditions, soil physical and chemical characteristics and radionuclide behavior in soil and plant.

In this context the relatively low transfer factors obtained in the present experiment could be probably explained by the following factors. Uranium is not an essential element for plant homeostasis. The availability of essential nutrients like calcium and potassium regulates the uptake of nonessential elements. The soil type under study was saturated with calcium exchangeable bases.

The availability of uranium for plant uptake from the soil solution was relatively low. Under oxidizing conditions U^{6+} is present in the form of the highly soluble uranyl ion UO_2^{2+} . It becomes even more soluble upon formation of uranyl carbonate complexes, under neutral to alkaline conditions in the soil solution. However despite the prevailing oxidizing conditions in tailings disposal sites, confinement and anaerobic bacteria activity may lead to reducing conditions resulting in precipitation of highly insoluble uraninite (Abdelouas, 2006). Coprecipitation with carbonate and phosphate (e.g. autunite, $Ca(UO_2)_2(PO_4)_2$) may also lead to uranium stabilization in mill tailings (Abdelouas et al., 1998).

Conclusion

Despite relatively high uranium activity concentrations of ^{234}U , ^{238}U and ^{235}U measured in soil, TFs of uranium isotopes determined in studied plants were relatively low and fell within the range of $n \cdot 10^{-3}$ ÷ $n \cdot 10^{-4}$.

Highest uptake of uranium isotopes was found in leafy vegetables, lower in cereals, and the lowest in corn. ^{234}U , ^{235}U and ^{238}U were accumulated with comparable transfer factors in leafy vegetables (sorrel, chard and arugula) - $n \cdot 10^{-3}$. In the case of corn, TF was one order lower - $n \cdot 10^{-4}$. TFs of cereals and sorrel were up to 3 times lower than these of chard and arugula.

Comparing TFs of uranium isotopes in barley varieties, up to 50% lower accumulation was found in barley F173 than in other two.

On the bases of TFs determined in the studied crops, corn, barley and triticale could be recommended for growing on the soils in the vicinity of the former uranium mine in Buhovo.

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Evaluation of Soil Oxide Mineralogy in Different Bedrock Lithologies in the Southern Guinea Savannah of Nigeria

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Abstract

Agriculture has very important social and economic footprint in Africa. Its soil resource is however threatened by degradation resulting from mismanagement, due to lack of knowledge on important soil functional properties such as mineralogy, which impact many soil processes. This study was therefore conducted to access variations in soil oxide mineralogy in soils formed from differing bedrock lithologies. Soil sampling was carried out on soils from three different bedrock types; basement complex rocks, recent alluvium, and Nupe sandstone in Kwara state, southern Guinea Savannah of Nigeria. Data was subjected to analysis of variance to determine significant differences in treatment means ($p < 0.05$), and correlation analysis was performed to determine relationships between measured variables.

Elemental oxide composition of soils from the different bedrock materials were found to be significantly different. Soils from all three bedrock types had very high amounts of silica by mass ($>80\%$). The highest mean percentage of 90.59 was recorded in recent alluvium-derived samples, while the least value of 80.75 % was recorded in basement complex-derived samples. Aluminum and iron oxides were the most saturating metal oxides in all the bedrock types, and significantly higher values of 5.65 % and 2.71 % for Al_2O_3 and Fe_2O_3 respectively were recorded in basement complex-derived soils. Dissimilar trends of mineral oxide relationships were observed in soils from the different lithologies, which are likely a result of bedrock characteristic, environmental and hydrologic factors. The relationships established between oxide minerals in this study could serve as a foundation for subsequent investigations into mineral interactions in the soils.

Keywords: soil management, oxide mineralogy, land, savannah

Introduction

Agriculture is the economic mainstay of more than 70 % of Africa's population, which impacts economic growth and social improvement, and forms a major trade component. This demonstrates the pivotal position of soil resources in the continent, both to keep up with food production for rapidly increasing populations, and for economic prosperity. Soils in this region however continue to be degraded due to poor management. According to the international fertilizer association (IFA, 2016), about 75 % of sub-Saharan

Africa (SSA) soils are already degraded. The soils have also been described as of limited resilience ability (Lal, 1995).

Rising populations in Africa implies more pressure on land resources for food production, which hangs majorly on soil fertility. Building soil fertility ordinarily implies increased use of nutrient inputs. Mineral fertilizers use in SSA soils is however constrained by high variability in responses to common standard fertilizers as a result of soil mineralogy variations, among other factors. For example, Ojanuga (1978) reported that most Nigerian soils are low in CEC and applied chemical fertilizer efficiency, due to the characteristic clay mineralogy of the soils. This leads to loss of mobile nutrients, nutrient deficiencies, and imbalance (Voortman, 2003).

High application of chemical fertilizers under this condition result in increased risk of nutrient elements and toxic chemicals contamination of surface and groundwater, and GHGs emissions which promote climate change. The situation is worsened when soil organic matter is depleted to critically levels (Vanlauwe et al., 2010). Optimum and sustainable soil management entails the generation of spatial information on soil functional properties such as nutrient capacity, toxic substances filtering, nutrient cycling and terrestrial gas fluxes, which are affected by factors such as texture and organic matter (OM) gradient, mineralogy, and elemental concentration.

Soil mineralogy is an important factor in crop selection and growth, and it has a direct effect on soil fertility, and the capacity of a soil to store and release nutrients for crop use (Ajiboye and Ogunwale, 2013). The extensive study of oxide and oxyhydroxide compounds in different soil types (Vandenbergh et al., 1986; Acebal et al., 2000; Stahr et al., 2010) highlights their importance in soil. According to Kinniburgh and Jackson (1981), soil adsorption is largely controlled by metal oxides and oxyhydroxides, and their level of coating on soil phyllosilicates. Iron oxides and other minerals whose structures are dominated by variable charge are major components in many acid soils such as those of the southern Guinea Savannah of Nigeria.

There is limited data on soil oxide mineralogical composition in Nigerian soils, which makes the study of their variation in different soils important. Knowledge of the mineralogical properties of the soils can help to better understand soil forming-factors and inherent functional properties, and inform the selection of management options that optimize yield, and conserve land resources for sustained agricultural productivity; an idea that is of utmost importance towards achieving food security in the SSA region. The foregoing formed the objective of conducting this study to improve the understanding of the relationships between soil bedrock type and oxide mineral composition in the studied soils.

Materials and Methods

This research was conducted in Kwara State, southern Guinea Savannah of Nigeria. Geographically, the state is located between latitudes 7°55' and 10° 05' N, and longitudes 2° 45' and 6° 15' E. The region has a tropical wet and dry climate, with double rainfall maxima (two peaks in July and September) and a dry spell in August. The dry season begins in early November, ending in early March. Both dry and wet seasons last for about six months. The state has an annual rainfall range of 1000 to 1500 mm, and atmospheric temperature is uniformly high (between 25 °C and 30 °C) throughout the wet season except between July

and August when clouding of the sky prevents direct insolation. In the dry season, temperature ranges between 33 °C to 34 °C (Olanrewaju, 2009). According to the federal department of agricultural land resources (FDALR) (1990), the geology of the state consists mainly of precambrian basement complex rocks (95 %), and recent alluvium and sedimentary (sandstone) rock materials (5 %). Olaniyan (2003) stated that the major metamorphic rocks found in the state are granitic gneiss, biotite gneiss, quartzite, augite gneiss and banded gneiss.

Free survey was carried out in three locations (Ilorin, Shonga and Patigi) within the state, informed by the FDALR (1990) soil map of Nigeria. According to the map, Ilorin falls within map unit 15g, with soils formed from basement complex rocks, Shonga falls on map unit 2a, having soils derived from recent alluvium, and Patigi is located on map unit 15e, with soils formed from Nupe sandstone. An area of 2 ha was marked out on fallow lands in each of these locations from which ten (10) surface (0-15 cm) soil samples were randomly taken. The soil sampling sites at Ilorin, Shonga and Patigi were located at approximately 8.450953° N, 4.658458° E; 9.012603° N, 5.172725° E; and 8.717586° N, 5.796669° E respectively (Figure 1). Soil samples were air-dried, <2 mm sieved, and subjected to physical and chemical analyses. Physical and chemical properties of soils were determined using routine laboratory procedures and presented in Table 1. Soils of the study location were classified according to USDA Soil Survey Staff guidelines (2014) as Anthraquic Ustorthent, Grossarenic Kandistalf and Ustic Quartzipsamment for Ilorin, Shonga and Patigi locations respectively.

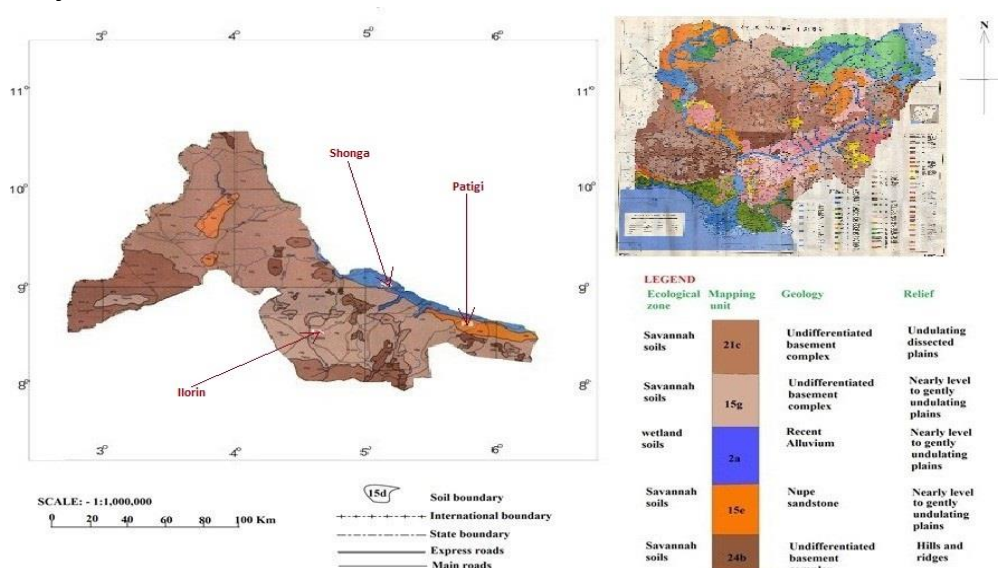


Figure 1. Soil map of Kwara state (extract from soil map of Nigeria (FDALR, 1990) indicating survey locations.

Quantitative determination of mineral element composition was done using X-Ray Fluorescence (XRF) spectroscopy analysis. Hundred grams (100 g) of soil samples were pulverized into a fine powder using a motorized agate mortar and passed through a 53 µm mesh screen. Two main analytical techniques were employed for the study; Energy Dispersive XRF (EDXRF) and atomic absorption spectroscopy (AAS). Loss on Ignition was determined by gravimetry using furnace method. Measured mineral oxide composition of the soils is presented in Table 2.

Table 1. Physico-chemical properties and variations in the A_p horizon of soils formed from three bedrock types.

Parameter	15g	2a	15e	LSD
Gravel (%)	3.92	5.22	4.85	1.681
Sand (%)	64.91	74.24	73.93	7.531*
Silt (%)	26.0	10.0	16.33	2.124**
Clay (%)	9.00	15.76	9.76	4.755*
pH	5.97	5.98	6.74	0.277**
Total N (%)	0.31	0.11	0.19	0.130*
P (mg/Kg)	1.46	3.10	2.43	0.43**
OC	0.21	0.47	0.54	0.088**
Ca ²⁺ (cmol/kg)	0.80	1.0	3.11	0.414**
Mg ²⁺ (cmol/kg)	0.33	0.28	1.17	0.310*
Na ⁺ (cmol/kg)	0.40	0.09	0.20	0.157*
K ⁺ (cmol/kg)	0.27	0.19	0.15	0.176
Exch. Acidity (cmol/kg)	0.10	0.13	0.1	0.042
ECEC (cmol/kg)	2.06	1.70	4.73	0.611**
BS (%)	94.83	91.98	97.88	1.408*

OC= organic carbon; ECEC= effective cation exchange capacity; BS= base saturation; LSD = Least significant difference; *= significant at $p<0.05$; **= significant at $p<0.01$

Table 2. Oxide mineral composition and variation in the A_p horizon of soils on three bedrock types.

Oxide	% composition			LSD
	15g	2a	15e	
SiO ₂	80.75	90.59	89.86	0.0360**
TiO ₂	2.07	1.57	1.2	0.004**
Al ₂ O ₃	5.65	2.19	2.2	0.025**
Fe ₂ O ₃	2.71	1.85	1.51	0.007**
Cl	1.0	-	-	0.047**
CaO	0.12	0.059	0.13	0.0003**
MgO	0.029	0.016	0.07	0.0005**
Na ₂ O	1.93	0.37	1.88	0.002**
K ₂ O	1.2	0.27	0.84	0.003**
MnO	0.077	0.049	0.059	0.0004**
V ₂ O ₅	0.028	0.015	0.009	0.0003**
Cr ₂ O ₃	0.016	0.012	<0.001	0.0005**
CuO	0.026	0.015	0.017	0.0005**
ZnO	0.003	-	-	0.0003**
Ga ₂ O ₃	0.015	-	-	0.0008**
As ₂ O ₃	0.007	-	0.01	0.0024**
Rb ₂ O	0.023	-	0.014	0.0017**
SrO	0.027	-	0.022	0.0009**
ZrO ₂	0.621	0.297	0.271	0.0007**
BaO	0.28	-	-	0.0003**
PbO	0.010	-	0.007	0.00006**
LOI	3.4	2.7	1.9	
Σ	99.992	100	99.999	

LOI: loss on ignition; LSD = Least significant difference; **=significant at $p<0.01$

Soil properties data was subjected to analysis of variance, and significant means were separated using the least significant difference (LSD). The relationship between oxide minerals was tested using Pearson's Correlation analysis performed with SPSS V20. Graphs were plotted using Microsoft Excel 2016.

Results and Discussion

Variations in mineralogical properties of soils

As expected, there was wide variation in elemental concentration between soils from the different bedrock types (Table 2). Higher levels of metal oxides TiO_2 , Al_2O_3 , Fe_2O_3 and MnO were recorded in basement complex bedrock (15g)-derived samples, and many of the trace elements found in the 15g samples were absent in samples from the other bedrock types. An example of these is Cl which was present only in 15g at 1 % weight by mass (Table 2). The graphical plot of oxide elemental composition in the soils is presented in Figure 2. Alkaline earth and alkali metal oxides also varied widely between the soils. These variations can be attributed to differences in parent material properties, hydrological and pedological factors between the sampling sites. SiO_2 (as a primary mineral) constituted the greatest percentage of the oxide mineral assemblage in all the soils, and the loss on ignition (LOI) values suggests that the soils were higher in mineral matter contents with low amounts of carbonaceous matter.

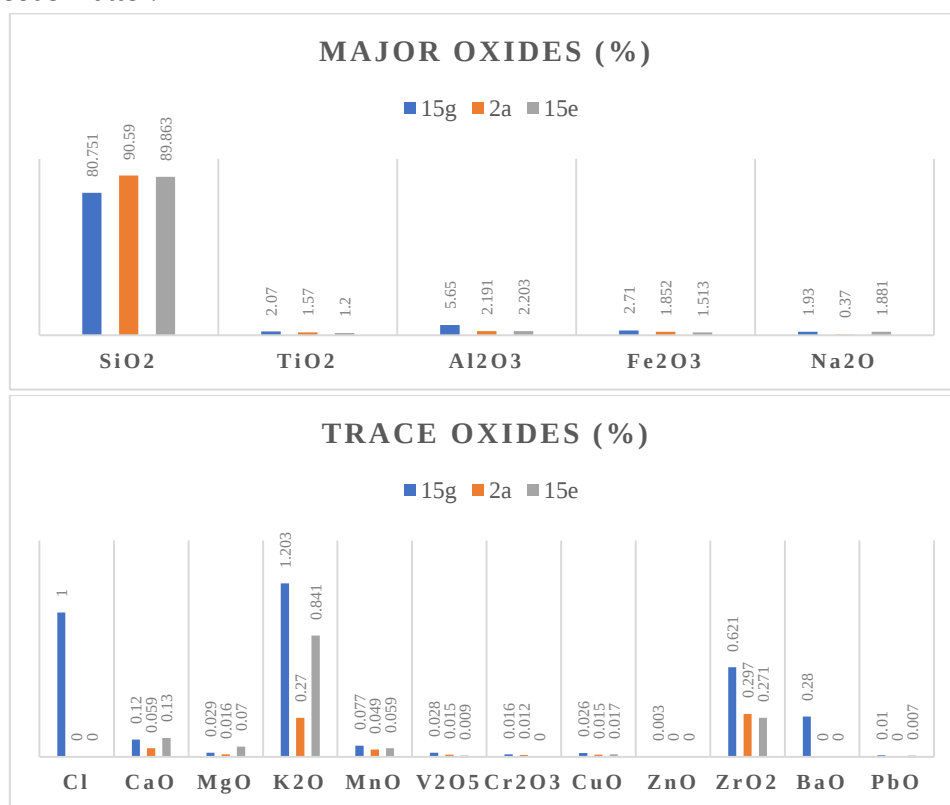


Figure 2. Mean oxide mineral composition of soils

The metal oxide composition trends in the soils in this study show that they have a high percentage of their primary rock minerals intact, signalling young weathering stages. According to Scheinost (2005), highly weathered soils that have lost a substantial amount of their Si, alkalis and alkaline earths are expected to contain high (up to 50 % by weight)

amounts of metal oxides. Somehow, LOI which is expected to be positively correlated to soil organic matter was lowest in 15e whose samples had higher OC content than 15g in which the highest LOI was recorded. There are no direct relationships from studied parameters to explain this observation. Significantly lower amounts of alkali and alkaline earth metal oxides were recorded in 2a samples (Table 2). This may be an indication that the clay in samples derived from this bedrock type allow more rapid soil water movement, leading to higher loss of the mineral under leaching conditions. According to Loughan (1969), while Al, Si, Ti and Fe oxides largely stay at the weathering site in the form of clay minerals, alkaline earth metals such as Ca are readily lost in soil under leaching conditions.

Relationships between major mineral oxides in soils

Tables 3, 4 and 5 show the correlation relationships between major oxide mineral elements in soils from the three bedrock lithology types. Trends and relationships between mineral oxides differed in the different bedrock lithologies (Tables 3-5). While relationships between Si, Ti oxides and alkali and alkaline earths were mainly positive in 2a and 15e, negative correlation relationships were recorded in 15g (Tables 3-5). In 2a, TiO_2 had a positive significant ($p < 0.01$) relationships with Na_2O ($r(10) = .999$) and K_2O ($r(10) = 1.00$). Na_2O and K_2O were also strongly positively correlated in this bedrock soils ($r(10) = 1.000$; $p < 0.01$) (Table 3). In 15e, positive significant ($p < 0.01$) relationships were recorded between TiO_2 and Al_2O_3 ($r(10) = .998$), Fe_2O_3 ($r(10) = .997$), CaO ($r(10) = .998$) and MgO ($r(10) = .997$).

Table 3. Oxide minerals relationships in Recent Alluvium (2a)-derived soil samples.

	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O	MnO	CuO	LOI
SiO_2											
TiO_2	.952										
Al_2O_3	.855	.655									
Fe_2O_3	.955	.817	.971								
CaO	.518	.756	.000	.240							
MgO	.877	.982	.500	.693	.866						
Na_2O	.952	.999**	.655	.817	.756	.982					
K_2O	.952	1.000**	.655	.817	.756	.982	1.000**				
MnO	.762	.924	.316	.535	.949	.980	.924	.924			
CuO	.952	.998**	.655	.817	.756	.982	.997**	.999**	.924		
LOI	.952	.997**	.655	.817	.756	.982	.997**	.998**	.924	1.000**	

** . Correlation is significant at the 0.01 level (2-tailed).

Al_2O_3 and Fe_2O_3 were also strongly positively correlated with CaO and MgO , and Ca and Mg oxides had a positive significant correlation ($r(10) = .998$; $p < 0.01$) (Table 4). In 15g, SiO_2 had a positive and significant relationship with TiO_2 ($r(10) = .998$; $p < 0.05$) and K_2O ($r(10) = .999$; $p < 0.05$) while negative significant relationships were recorded with CaO , MgO , MnO , CuO and ZnO . Strong ($p < 0.01$) negative relationships were also recorded between TiO_2 , CaO , MgO , MnO and ZnO . Cl which was detected only in 15g soil samples had a strong correlation with Fe_2O_3 ($r(10) = 1.000$; $p < 0.01$).

These analyses show that Si, Ti, alkali and alkaline earth metal oxide relationships were majorly positive in 2a and 15e, while negative relationships were largely recorded in 15g. These geochemical characteristics and trends can be attributed to bedrock lithology imprint on the examined soils. Orescanin et al. (2009) reported similar oxide minerals relationships recorded in 15g, while the trends in 2a and 15e are not in consonance with the findings of their research.

Table 4. Oxide minerals relationships in Nupe Sandstone (15e)-derived soil samples.

	SiO ₂	TiO ₂	Al ₂ O ₃	Fe ₂ O ₃	CaO	MgO	Na ₂ O	K ₂ O	MnO	CuO	PbO	LOI
SiO ₂												
TiO ₂	.682											
Al ₂ O ₃	.682	.998**										
Fe ₂ O ₃	.682	.997**	.998**									
CaO	.682	.998**	.999**	.997**								
MgO	.682	.997**	1.000**	.999**	.998**							
Na ₂ O	.956	.894	.866	.867	.885	.866						
K ₂ O	.974	.500	.566	.500	.511	.630	.866					
MnO	.929	.904	.911	.926	.903	.966	.997	.822				
CuO	-.731	0.000	.000	.000	.000	0.000	-.500	-.866	-.427			
PbO	-.468	.327	.387	.343	.327	.357	-.189	-.655	-.108	.945		
LOI	-.292	.500	.500	.500	.500	.500	0.000	-.500	.082	.866	.982	

Table 5. Oxide minerals relationships in Basement complex (15g)-derived soil samples.

	SiO ₂	TiO ₂	Al ₂ O ₃	Fe ₂ O ₃	Cl	CaO	MgO	Na ₂ O	K ₂ O	MnO	CuO	ZnO	PbO	LOI
SiO ₂														
TiO ₂	.998*													
Al ₂ O ₃	.984	.993												
Fe ₂ O ₃	-.896	-.866	-.803											
Cl	-.896	-.867	-.803	1.000**										
CaO	-.998*	-1.000**	-.993	.866	.864									
MgO	-.998*	-1.000**	-.995	.861	.866	1.000**								
Na ₂ O	-.987	-.974	-.942	.956	.956	.974	.974							
K ₂ O	.999*	.998*	.990	-.879	-.879	-.998*	-.998*	-.980						
MnO	-.998*	-1.000**	-.993	.826	.863	.999**	.999**	.974	-.998*					
CuO	-.998*	.999**	-.992	.886	.856	1.000**	.999**	.972	-.999*	1.000**				
ZnO	-.998*	-.999**	-.993	.866	.866	.999**	.998*	.970	-.999*	.999**	.999**			
PbO	-.896	-.866	-.803	.999**	1.000**	.866	.887	.956	-.879	.866	.866	.866		
LOI	.554	.500	.397	-.866	-.866	-.500	-.570	-.682	.524	-.500	-.500	-.500	-.866	

**Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed)

Generally, oxide minerals distribution and relationships in the soils point to the effect of bedrock materials on resulting soil mineralogical properties, and the likelihood of differing

bedrock permeability and drainage type, which favours higher base saturation in 15g and 15e than in 2a (Table 1). The influence of differing weathering mechanisms on soil mineralogy in different bedrock types have also been reported in previous research including Miko et al. (2001).

Conclusion

Results from this study highlighted marked differences in analytical values of oxide mineralogy in the soils examined, and suggests a likely impact of bedrock lithology on recorded geochemical properties. This is more so, as weather and climate characteristics are similar for all three locations examined. Soils formed from recent alluvium had higher quartz component and lower amounts of Al, Mn and Ti oxides which may be a result of depletion owing to the weathering mechanism in the bedrock material. The low Ca and Mg oxides and base saturation in soil samples derived from the recent alluvium bedrock material also suggests that the clay composition of this bedrock type is more prone to nutrient leaching losses than those of the other bedrock types. Further studies covering a wider land area and analytic approaches will be required to generalize the findings from this study.

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Growth and Development of *Paulownia tomentosa* and *Paulownia elongata* x *fortunei* in Glasshouse Experiment

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Abstract

The growth potential of *Paulownia tomentosa* and *Paulownia elongata* x *fortunei*, cultivated at three planting densities in a greenhouse was investigated, using conventional field and laboratory methods. Data on the survival percentage, base diameter, total plant height, biomass and leaf area were obtained and analyzed. Differences in growth, productivity, survival and biomass allocation pattern of the tested clones of *Paulownia tomentosa* and *Paulownia elongata* x *fortunei* were found. The results obtained showed that hybrids of *Paulownia elongata* x *fortunei* do not exceed the growth performance of the *Paulownia tomentosa* species for the region of Sofia.

Key words: fast-growing forest trees, plant height, base diameter, biomass allocation

Introduction

Over the last two decades, species and hybrids of genus *Paulownia* have attracted the attention of many researchers, mainly due to the fact that they are fast-growing and suitable for testing at higher planting densities, at short-rotations, and possess potential for repeatable reproduction. According to the literature (Ericsson and Nilsson, 2006), short-rotation plantations will be increasingly used for biomass production in the future, and species or clones with high repetitive regenerative capacity will be particularly valuable. Moreover, Maier and Vetter (2004) reported that, unlike most fast-growing tree species, *Paulownia tomentosa* increases its bioproductivity during the second rotation or after the first 4-year cycle. Advantageous for using hybrid *Paulownia* clones in agroforestry or short rotation cultivation are characteristics such as drought tolerance, unpretentiousness to soil composition, tolerance to wide daily temperature amplitudes, which according to some authors (Tang et al., 1980), makes them in demand on the world market. In contrast to *Paulownia tomentosa*, which is introduced in the early 20th century and is already naturalized, *Paulownia elongata* x *fortunei* have been tested in Europe just recently (Zuazo et al., 2013; García-Morote et al., 2014). In Bulgaria, the first experiments started in 2004 with the development of *in vitro* propagation protocol. The first experimental plantations for bioproductivity studies on different terrains and in different parts of the country were initiated twelve years ago (Gyuleva, 2008, 2010; Gyuleva et al., 2012; Gyuleva et al., 2013; Gyuleva, 2014; Stankova et al., 2016, 2019).

The growth and development of tree species at a young age in response to the available growing space (number of plants per unit area) is a key aspect in the ecology of trees in forest ecosystems and forest plantations. Higher density reduces light, food and water availability, which has an effect on each individual. The phenotypic changes that occur are expressed by such measurable characteristics as plant height and diameter, and the biomass partitioning to various organs - stem, branches, leaves and roots (Longbrake and McCarthy, 2001; Sinacore et al., 2017). The first results in Bulgaria to model the production of stem biomass at an early age in *Paulownia*, depending on the diameter of the plant, are already known (Stankova et al., 2016). The analysis of the localization of plant biomass in tree organs such as stems, branches, leaves and roots is considered a modern ecological approach to assess the adaptability of the tree species to site conditions (Poorter et al. 2012, 2015). In our country, such an analysis has not been conducted so far.

The aim of the present study is to evaluate and compare growth and productivity parameters of *Paulownia tomentosa* and *Paulownia elongata* x *fortunei* at different ages and stocking levels, and to analyze the biomass localization to leaves, stems and root system during two-year cultivation at uncontrolled greenhouse conditions.

Materials and Methods

At the beginning of April 2017, 500 isolated root cuttings of *Paulownia tomentosa* (clone group based on isolated roots of half-sib saplings) and *Paulownia elongata* x *fortunei* (clone group based on three clones) were planted in flower pots. The roots were obtained from experimental four-year-old plantations, established on the territory of Strumyani, (Mikrevo nursery) and Zlatna Panega. Alluvial soil, enriched with manure, was used (pH= 7.2 and low N content before enrichment). After two months of cultivation, the newly developed plants were replanted in 7-liter containers and arranged in a scheme, providing three variants of growing space: 0.018 m², 0.027 m² and 0.053 m² of growing area in one container, respectively. The pots were placed in greenhouse beds filled with 15 cm - layer of sand. The depth of each container was 18 cm. The number of containers for each variant was 12, and the numbers of the measured plants were 12, 24 and 36, respectively. The surviving plants of the different variants were replanted in 20-liter containers with 28 cm of depth in April 2018, for further measurements. The newly provided area was 0.024 m², 0.035 m² and 0.071 m², respectively. Biometric measurements were performed with an electronic caliper and a metal roulette. The electronic scale Mettler - Toledo with an accuracy of 0.00001 g was used to measure the fresh weights *in situ*. The oven-dried weight (g) was obtained using the moisture content of the obtained stem, leaf and root samples. Individual plant leaves were isolated from each variant, scanned and measured using AutoCad2014 and a scaling factor of 0.01 to define leaf area, leaf length and leaf width.

The experiment was conducted in two stages. During the first growing season, height, diameter, leaf area per tree per variant were analyzed three times (June, July and October), and survival during the winter season was assessed. During the second stage, the biomass allocation by fractions (root, stem and leaves) was studied in order to evaluate the environmental adaptability of the two clonal populations and to estimate specific leaf area (cm²/g), leaf area ratio (LAR) and Root-Shoot-Ratio (RSR).

Analysis of variance (ANOVA) with main factors of growth (height and diameter): age (month), clonal population and growing space was applied during the first study stage. Linear regression analysis to study the relationship between biomass fractions and measurable quantitative characteristics, such as diameter at the base and height of the plant, was used during the second stage of the study.

Statistical data processing was performed using software packages R “stats” and “agricolae” (R Core Team 2019; de Mendiburu 2020), and the visualization of the data was carried out with graphical functions of the R package “ggplot2” and “dplyr” (Wickham, 2016; Wickham et al., 2020).

Results and Discussion

The diameter at the base of the plant stem for the two clonal populations was measured three times in 2017 (Fig.1), and an analysis of variance (ANOVA) was applied (Table 1).

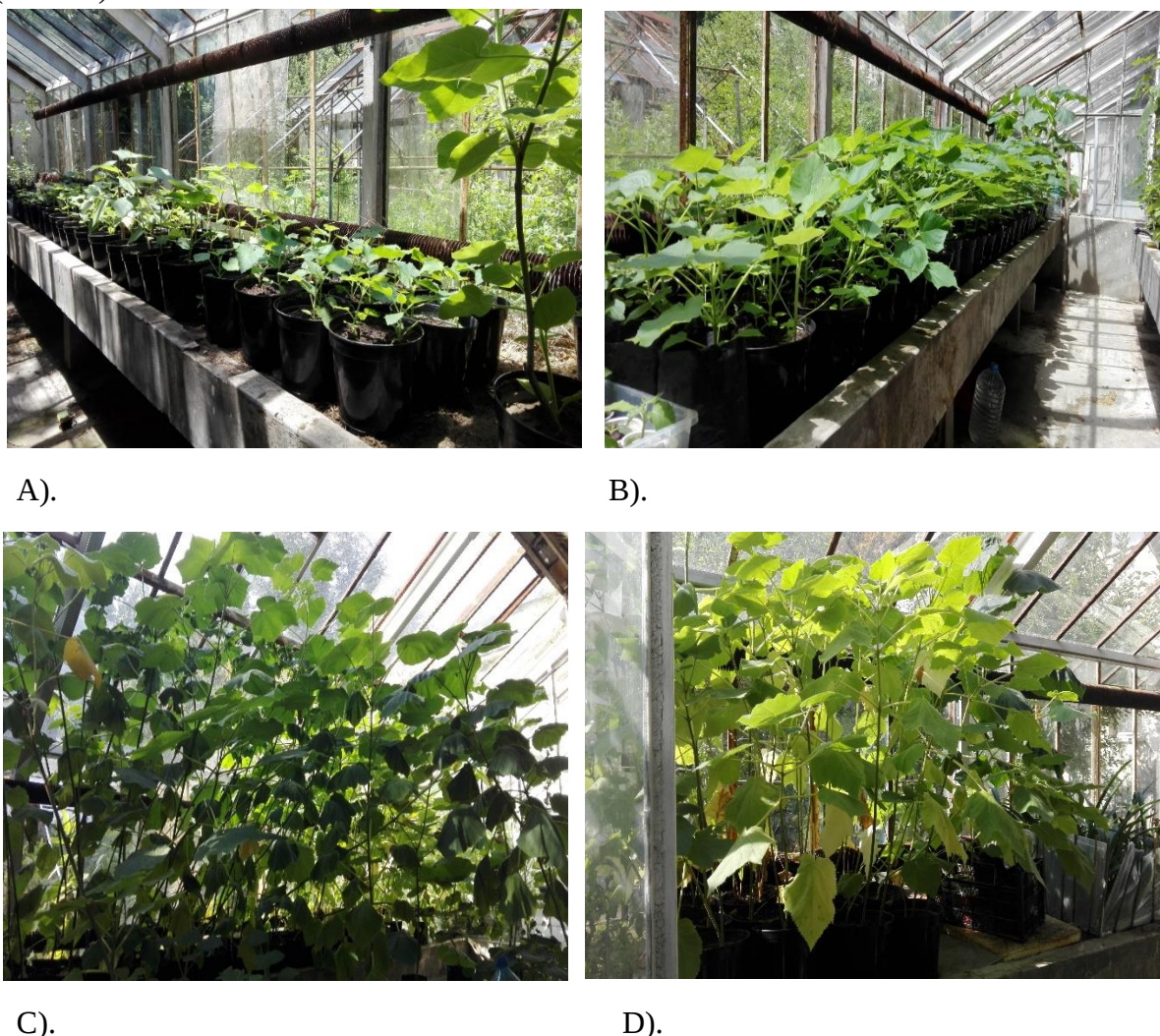


Fig. 1. Measurements during season 2017: A). *P. tomentosa* and *P. elongata* x *fortunei*- June 2017; B). Intermediate measurement – July 2017; C) *P. elongata* x *fortunei* – September 2017; D) *Paulownia tomentosa* – September 2017 before the last measurement.

ANOVA identified significant variation in the base diameter between the two clonal populations ($F = 9,272$ $df = 1$, p -value: 0,003) in the first studied month. At this early stage of the experiment, the base diameter of the clonal population of *Paulownia tomentosa* was significantly higher than that of the hybrid *Paulownia elongata* x *fortunei* in all three tested variants of growth area (Fig.2).

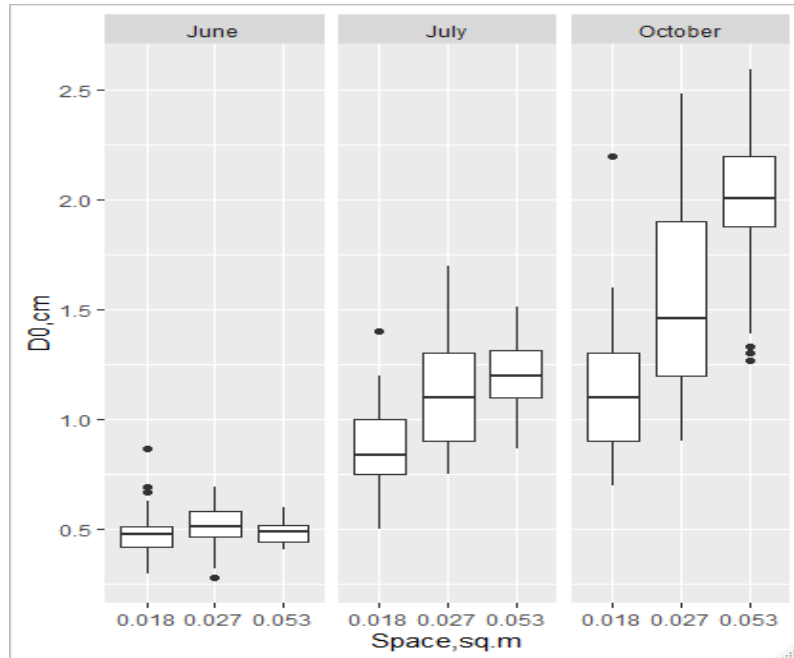


Fig. 2. Effect of growing space (m^2) on the base diameter $D0$ (cm) of paulownia plants according to age (month of measurement 2017).

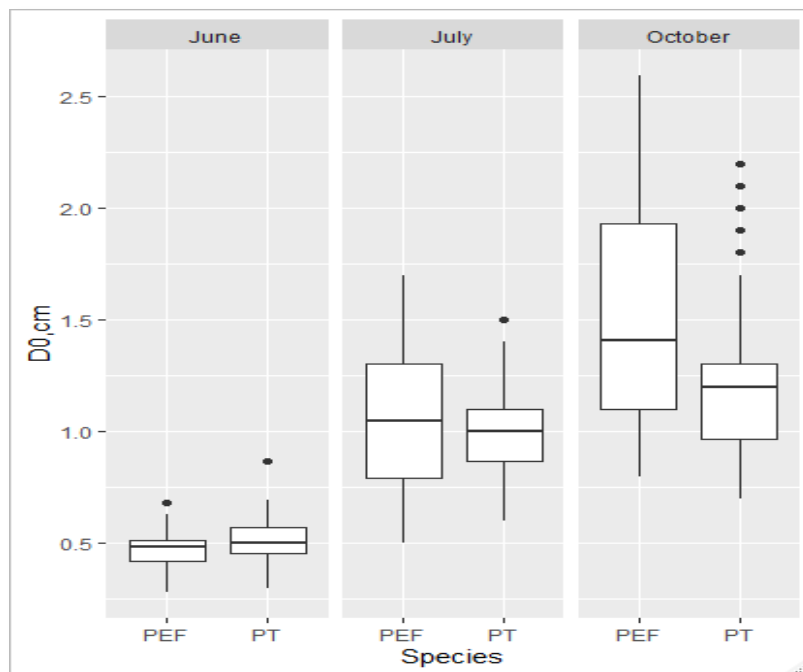


Fig. 3. Effect of the clonal population (*Paulownia tomentosa* (PT) and *Paulownia elongata* x *fortunei* (PEF)) on the base diameter $D0$ (cm) according to age (month of measurement 2017).

The factor growing space at this early stage has no significant effect ($F = 2.636$ and $df=2$, p -value: 0,075). With an average minimum temperature of 4.8°C and an average maximum temperature of 16.6°C for the preceding April, and an average minimum temperature of 9.5°C and an average maximum temperature of 20.5°C for the preceding May, this difference in favor of *Paulownia tomentosa* is not surprising. In our opinion, the main reason is the species differences between the two clonal populations. *Paulownia tomentosa* tolerated significantly better than *Paulownia elongata* x *fortunei* the lower temperatures in April and May. As the growing season progressed, the effect of the growth area as a factor increased (Fig. 1), and the effect of the clonal group weakened (Table 1).

Table 1. Analysis of Variance for the variable base diameter (D_0 , cm) as affected by factors Growing space and Clonal population

Month	Factors	Df	Sum Sq	Mean Sq	F value	Pr(>F)
June	Growing space	2	0,1547	0,0773	2,636	0,075
	Clonal population	1	0,2720	0,2720	9,272	0,003**
	Growing space X Clonal population	2	0,1483	0,0741	2,527	0,085
	Residuals	138	4,0489	0,0293		
July	Growing space	2	3,2601	1,6301	47,238	2,353e-16***
	Clonal population	1	0,1980	0,1980	5,739	0,018*
	Growing space X Clonal population	2	1,2530	0,6265	18,156	1,000e-07***
	Residuals	138	4,7620	0,0345		
October	Growing space	2	6,8738	3,4369	81,247	2,2e-16***
	Clonal population	1	1,4275	1,4275	33,474	4,140e-08***
	Growing space X Clonal population	2	0,9505	0,4753	11,235	3,016e-05***
	Residuals	138	5,8377	0,0423		

Significance level: '***' – $P < 0,001$ '**' – $P < 0,01$ '*' – $P < 0,05$

At an average minimum temperature of 14.3°C and an average maximum temperature of 27.1°C for the anterior June, *Paulownia elongata* x *fortunei* produced in July lower base diameter only at the variant of growth area of 0.027 m^2 , compared to *Paulownia tomentosa*. Under the conditions of the other two variants of growing space *Paulownia elongata* x *fortunei* produced base diameter superior to that by *Paulownia tomentosa* and the differences were statistically significant. At the end of the growing season, the influence of the two factors growth area and clonal group, as well as the interaction between them, was clearly expressed (Table 1). At an average minimum temperature of 15.6°C and an average maximum temperature of 29.0°C for the preceding August, and an average minimum temperature of 11.5°C and an average maximum temperature of 23.6°C for the preceding September, *Paulownia elongata* x *fortunei* continued to produce significantly higher stem base diameter than that of *Paulownia tomentosa* (Fig. 3).

The effect of the growing space as a factor for the variable total tree height was also studied during the first growing season (Table 2).

Table 2. Analysis of Variance for the variable height (H , cm) as affected by factors Growing space and Clonal population.

Month	Factors	Df	Sum Sq	Mean Sq	F value	Pr(>F)
June	Growing space	2	1899,63	949,81	70,363	2,2e-16***
	Clonal population	1	266,83	266,83	19,767	1,784e-05***
	Growing space X Clonal population	2	1553,91	766,96	57,557	2,2e-16***
	Residuals	138	1862,83	13,50		
July	Growing space	2	177849	88925	188,205	2,2e-16***
	Clonal population	1	5700	5700	12,064	0,001***
	Growing space X Clonal population	2	39	19	0,041	0,960
	Residuals	138	65203	472		
October	Growing space	2	198347	99173	431,124	2,2e-16***
	Clonal population	1	12916	12916	56,150	7,304e-12***
	Growing space X Clonal population	2	68	68	0,297	0,744
	Residuals	138	230	230		

Significance level: '***' – $P < 0,001$ '**' – $P < 0,01$ '*' – $P < 0,05$

ANOVA identified significant variation in the height, depending on the growing space, in June ($F = 70.363$ and $df=2$, p -value: $< 2.2e-16$). The maximum height was registered at the intermediate variant of stocking - $0,027 \text{ m}^2$ (Fig. 4). Its value was significantly higher than those obtained for the other two variants of growing space.

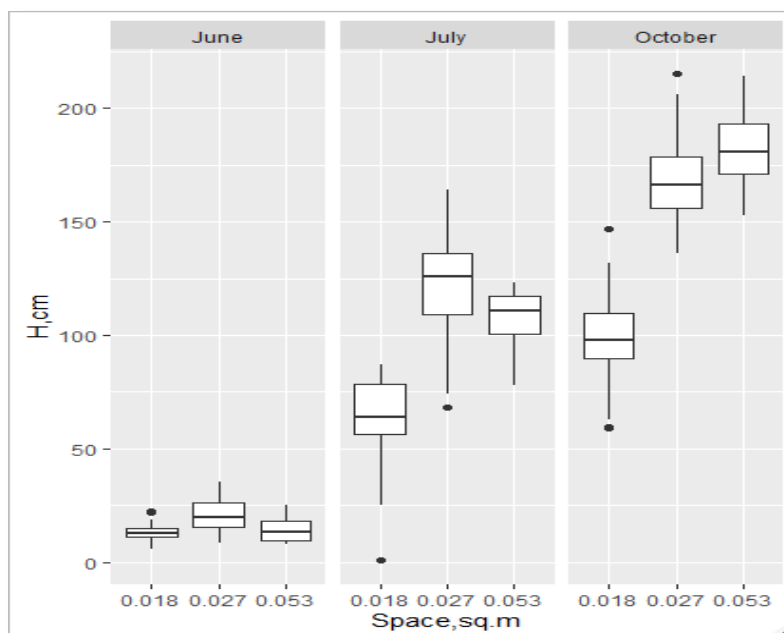


Fig. 4. Effect of the growing space (m^2) on the height H (cm) of paulownia plants according to age (month of measurement 2017).

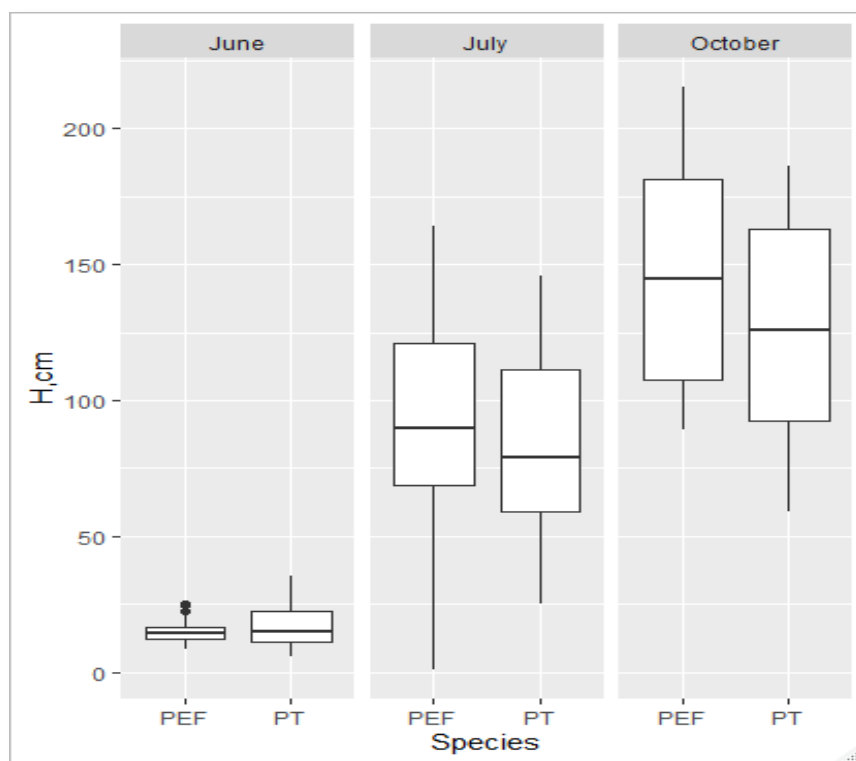


Fig. 5. Effect of the clonal population (*Paulownia tomentosa* (PT) and *Paulownia elongata* x *fortunei* (PEF)) on the height *H* (cm) according to age (month of measurement 2017).

Also, ANOVA identified high variation in height, depending on the clonal population ($F = 19.767$ and $df=1$, p -value: $1.784e-05$). The difference in the values of the heights was again statistically significant and was in favor of *Paulownia tomentosa* (Fig. 5).

The temperature conditions mentioned earlier should be considered for this result as well. In July, ANOVA identified significant variation in heights depending on both factors, growing space ($F = 188.205$ and $df=2$, p -value: $2.2e-16$) and clonal population ($F = 12.064$ and $df=1$, p -value: 0.00069). There was no indication for significant interaction between them. The maximum value of the height was registered again under the conditions of the second variant of stocking – 0.027 m^2 (Fig.4). This result, as well as the result of the first measurement in June, showed that individuals compete successfully with each other. In addition, all mean height values obtained differed significantly from each other, under the influence of the available growing space. At this stage, *Paulownia elongata* x *fortunei* is already taller than *Paulownia tomentosa* and the difference in the heights of the clonal populations was statistically significant (Fig.5). In October, ANOVA identified again a significant variation for this parameter, both for the growing space factor ($F = 431.124$ and $df=2$, p -value: $2.2e-16$) and for the clonal population factor ($F = 56.149$ and $df=1$, p -value: $7.304e-12$). The interaction between the two factors was absent. At this stage, the effect of the growing space is tangible. The maximum average height was reached by the two clonal populations at the largest available growing space – 0.053 m^2 . As the growing space decreased, the average height also decreased and the differences between the three studied variants were statistically significant. *Paulownia elongata* x *fortunei* again exceeded the mean height of *Paulownia tomentosa* by 18.94 cm.

More detailed study of the variation of the base diameter of the plant and its height with age and growing space showed that the two clonal populations follow the same pattern of growth and development, but both factors have a differentiated effect on the studied clonal populations (Figures 6 and 7).

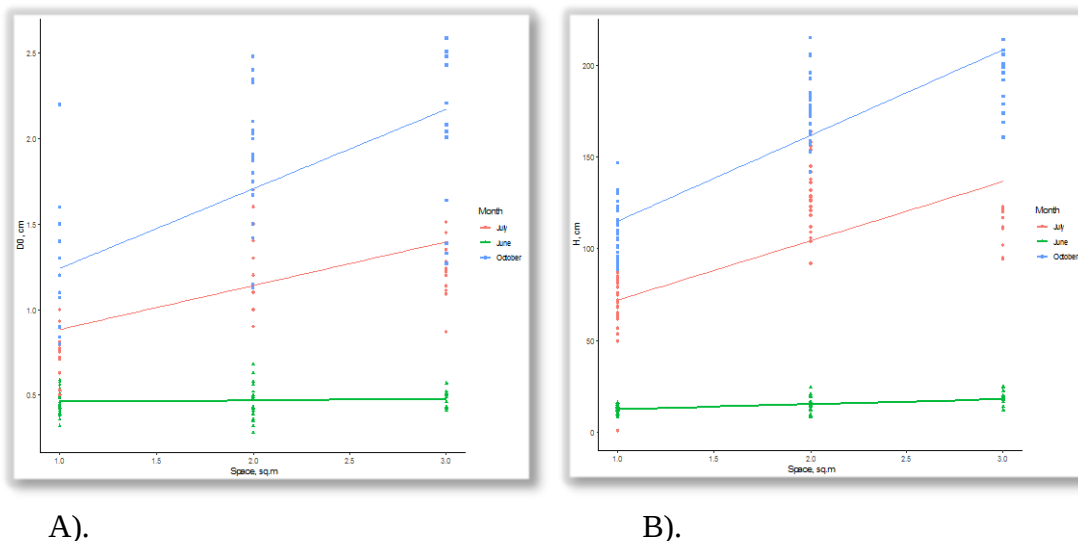


Fig. 6. Response of the base diameter D_0 , cm (A) and height H , cm (B) of *Paulownia elongata x fortunei* to the three spacings tested during the growth period June - October 2017.

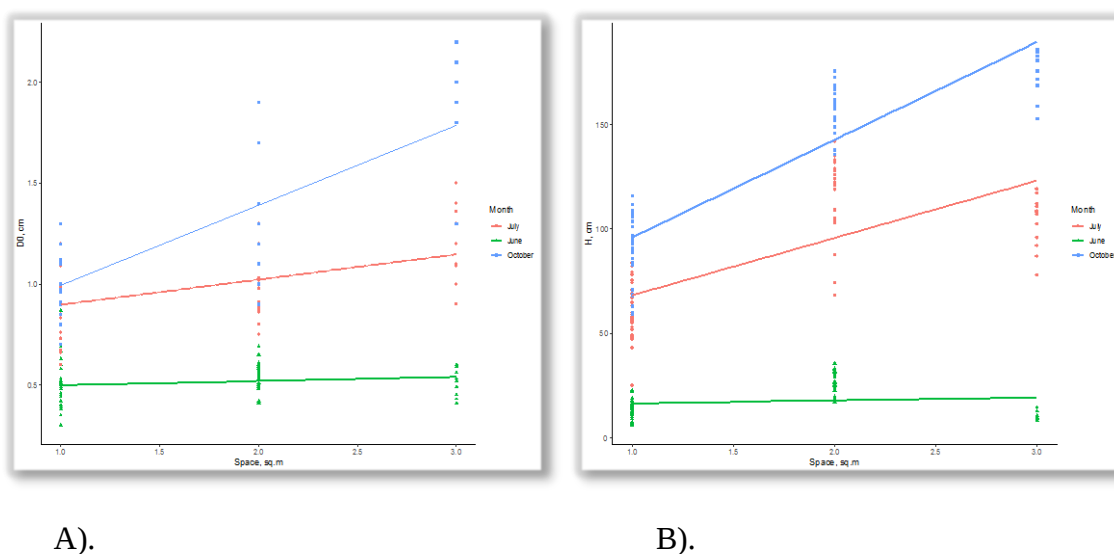


Fig. 7. Response of the base diameter D_0 , cm (A) and height H , cm (B) of *Paulownia tomentosa* to the three spacings tested during the growth period June - October 2017.

The positive response of age on the base diameter and height was apparent and explained more 70% of the variation in all cases. The response to the applied growing space was obvious too, but for the clonal population of *Paulownia elongata x fortunei* the smallest growing space differed for both parameters. The effect of the growing space becomes more noticeable with increasing of age (figures 2, 4, 6, 7) due to the larger sizes of the plants, which require larger growth area for their development. The growing space as a factor explained more than 15% of the variation in the height and less than 10% of the variation in the base

diameter. The base diameter increased in proportion to the growing space but total height reached maximum values under the conditions of the second variant – 0.027 m². The last is in agreement with the findings of Panetsos (1980) for the growth pattern in different poplar genotypes. It was clearly demonstrated that the maximum values of tree heights were reached at the intermediate growth space.

For the period studied, the clonal population of *Paulownia tomentosa* completed its growth phase earlier. The yellowing of the leaves and their final fall ended by the end of October, when the average minimum temperature for the month was 6.57⁰C, and the maximum average was 19.37⁰C (Figure 1). The clonal population of *Paulownia elongata* x *fortunei* completed its growth phase with a month delay, when the mean minimum temperature was 2.2⁰C and the mean maximum temperature was 10.1⁰C. The last leaves fell in early December. Compared to *Paulownia tomentosa*, *Paulownia elongata* x *fortunei* required longer time to enter dormant state, which at the climatic conditions for the region of Sofia, is an unfavorable feature.

At the end of the growing season of 2017 was found that the number of leaves per tree in the *Paulownia elongata* x *fortunei* hybrids at spacings 0.053 m² and 0.027 m² was 18. The average number of leaves per stem at the smallest space - 0.018 m² was 9.83. The leaf area, after an increase under the conditions of the intermediate variant, where it reached 342.54 cm², substantially decreased under the conditions of the smallest growing space - 47.084 cm². The mean number of leaves per stem of *Paulownia tomentosa* decreased from 15 to 9.25 from the larger to the smaller growing space. The same trend was found for the leaf area of *Paulownia tomentosa* - from 450,102 cm² at growing space 0.053 m², the leaf area decreased to 210.297 cm² at the growing space 0.018 m². The large size of the leaves, developed on *Paulownia tomentosa* at the growing space 0.053 m² was impressive. Under suitable growing conditions, the presence of large leaves in the first year is very distinctive of *Paulownia* species (Zhu et al., 1986).

The assessment of the survival rate of *Paulownia elongata* x *fortunei* during the relatively mild and warm winter (2017-2018) showed that at the spacings of 0.027 m² and 0.018 m² the percentage of frozen stems was 25% and 75%, respectively. The survival rate of *Paulownia tomentosa* per stocking variant was quite similar, but the percentage of the frozen stems differed and varied from 20% to 47%. The main difference, observed between the two clonal populations, was that the frost injuries of the stems of *Paulownia tomentosa* at all three tested spacings were partial, while in *Paulownia elongata* x *fortunei* the frost injuries of the stems were complete. The experiment clearly demonstrated that plants with a base diameter less than 1 cm did not survive after the end of the winter. The reported lowest minimum temperatures for January 2018 and February 2018 were -12.7⁰C and -10.3⁰C, respectively. The maximum temperatures for the same months were 13.6⁰C and 16.2⁰C, respectively. Under this temperature regime, the root systems of the both populations remained viable and after the cutting of the brownish (frozen) stems, retained their regenerative capacity to produce new shoots in the next growing season.

At the beginning of August 2018, various plants originated from the three growing space were subjected to destructive sampling in order to study the localization of the plant biomass to leaves, stems and roots (Fig. 8).

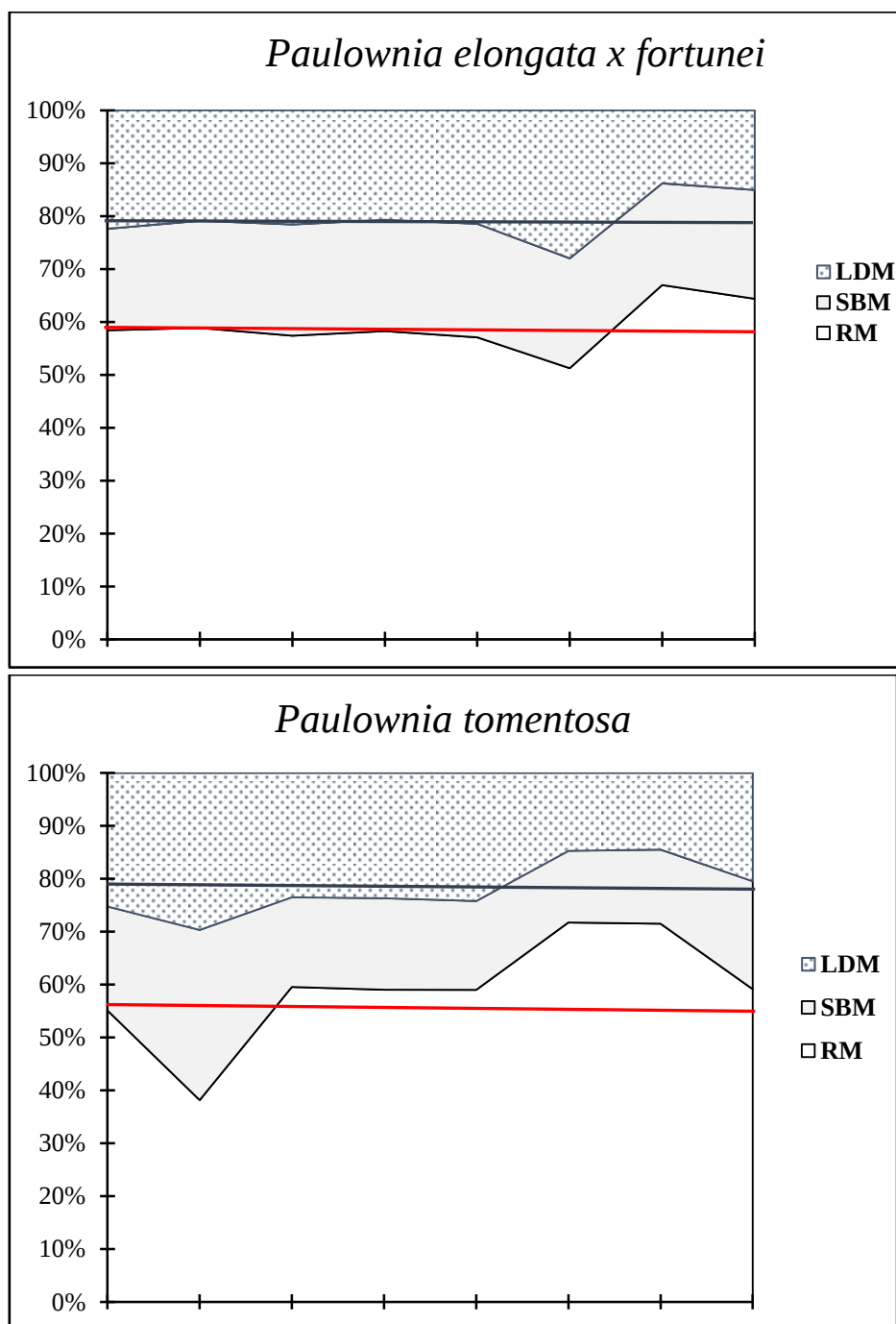


Fig. 8. Oven-dry biomass allocation (%) in leaves (LDM), stems (SBM) and roots (RM) of *Paulownia elongata x fortunei* and *Paulownia tomentosa* clonal populations.

During the study period, both clonal populations accumulated the largest amount of dry biomass in their root system. The largest fraction of the root system, as compared to the stem and leaves, has already been observed for *Paulownia tomentosa*, grown at different light gradients (Longbrake and McCarthy, 2001). The main difference that discriminated *Paulownia elongata x fortunei* and *Paulownia tomentosa* in our study was the amount of dry biomass accumulated in the stem and the leaves. At this early stage, *Paulownia tomentosa* accumulated more leaf dry mass, while *Paulownia elongata x fortunei* accumulated more stem mass.

In fact, such differentiated biomass localization indirectly explained the better growth performance of *Paulownia elongata*, as well as its hybrids with *Paulownia fortunei*, that has already been reported in the literature (Zhu et al., 1986). For the climatic and light conditions of Sofia, however, the hybrid *Paulownia elongata* x *fortunei* failed to realize its full growth potential. There was a strong restraining effect of the environment.

The relationship between different biomass fractions and measurable quantitative characteristics, such as base diameter, plant height and leaf area, was analyzed. The linear relationship between root mass and leaf area in the two clonal populations was studied. The linear regression model showed that the accumulation of dry biomass in the root system leads to an increase in leaf area (Fig. 9).

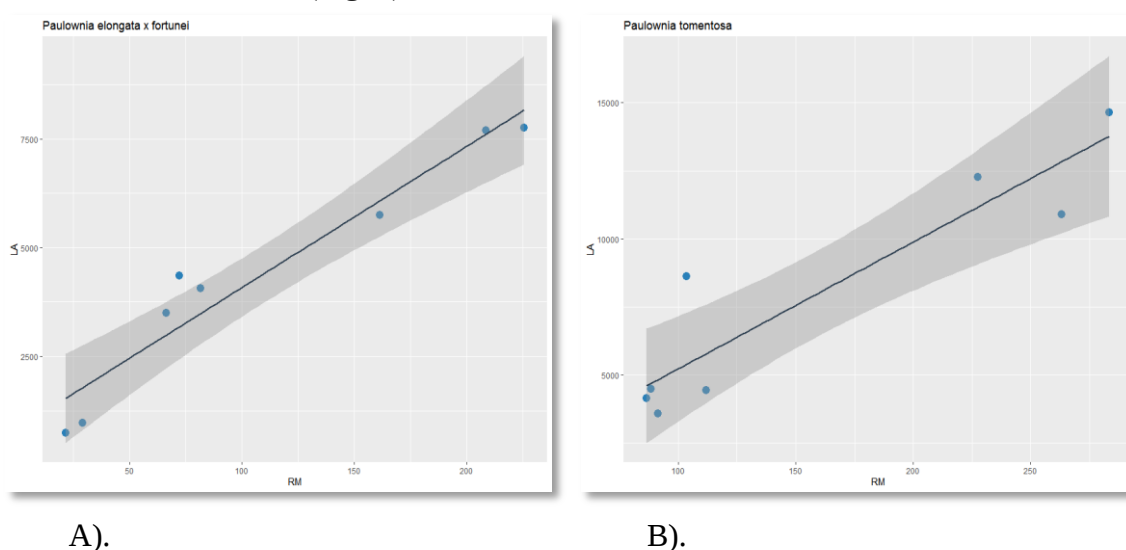


Fig. 9. Linear relationship between root biomass (RM) and leaf area (LA) observed for *Paulownia elongata* x *fortunei* (A) and *Paulownia tomentosa* (B). The gray bands around the line represent the standard error of the regression line.

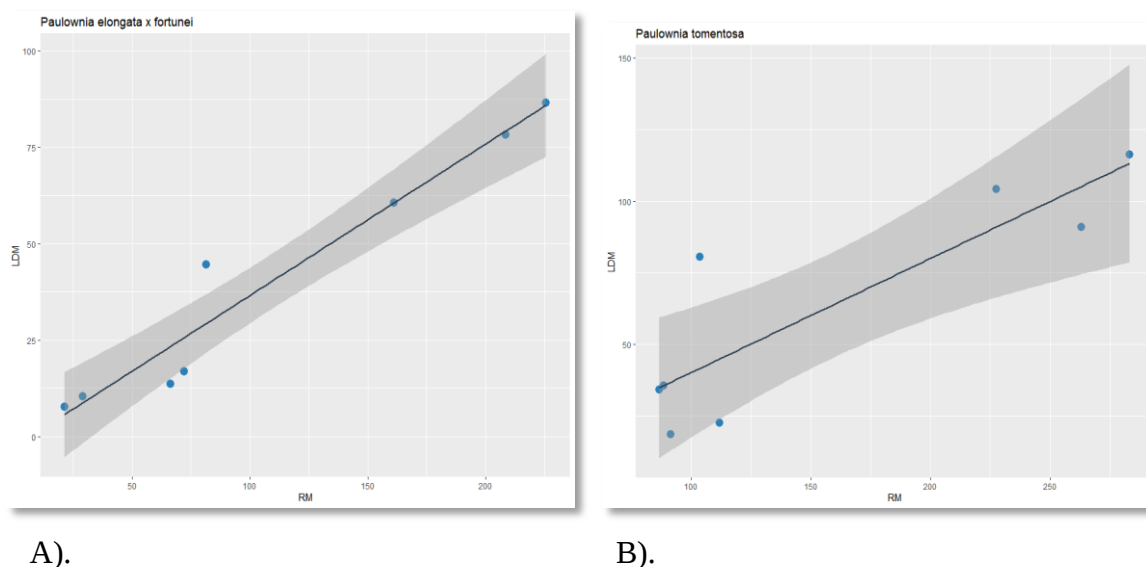
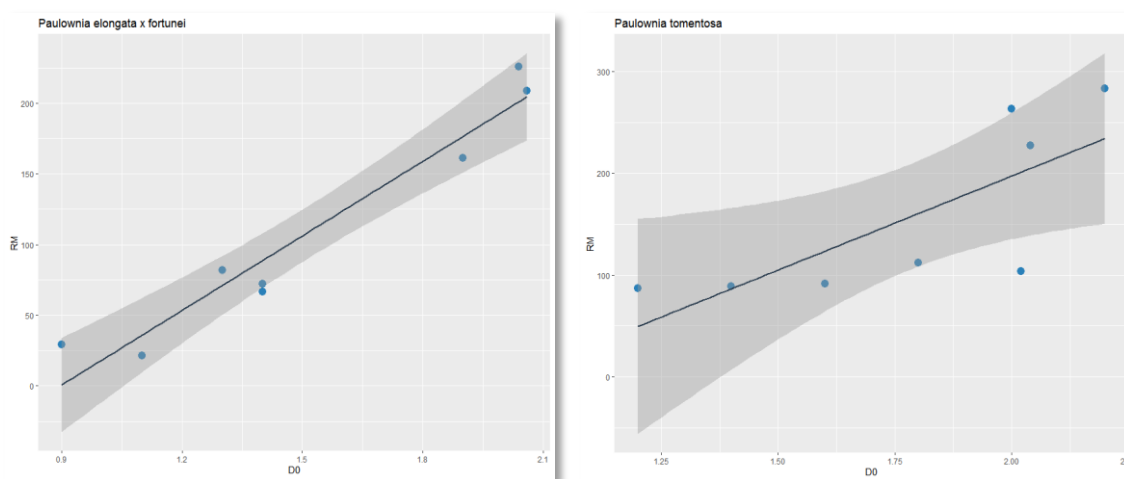


Fig. 10. Linear relationship between root biomass (RM) and leaf mass (LM) observed for *Paulownia elongata* x *fortunei* (A) and *Paulownia tomentosa* (B). The gray bands around the line represent the standard error of the regression line.

This relationship is significantly better expressed in *Paulownia elongata* x *fortunei* ($F = 78.75$ on 1 and 6 = df, $p = 0.000114$ and adj. $R^2 = 0.9174$) than in *Paulownia tomentosa* ($F = 33.5$ on 1 and 6 = df, $p = 0.0012$ and adj. $R^2 = 0.8228$), but in the last the leaf area increases faster (coefficient for the slope of the lines 46.63 vs. 32.45).

The regression analysis showed also that the accumulation of root biomass leads to an increase in leaf mass (Fig. 10). This relationship is again better expressed in *Paulownia elongata* x *fortunei* ($F = 99.9$ on 1 and 6 = df, $p = 5.809\text{e-}05$ and adj. $R^2 = 0.9339$) than in *Paulownia tomentosa* ($F = 17.8$ on 1 and 6 = df, $p = 0.005569$ and adj. $R^2 = 0.7059$). At the same weight of the root system, *Paulownia tomentosa* maintains a larger amount of leaf mass (values for intercept and slope of the lines 0.418 and 0.398 vs -2.729 and 0.393). These results indicate that root mass is a good predictor of the leaf mass and leaf area in both clonal populations tested, which has already been demonstrated in *Populus tremuloides* (Caldwell and O'Hara, 2017).

Linear regression analysis also showed that as the base diameter increased, so did the root dry mass (Fig. 11).



A).

B).

Fig. 11. Linear relationship between base diameter (D_0) and root mass (RM) observed for *Paulownia elongata* x *fortunei* (A) and *Paulownia tomentosa* (B). The gray bands around the line represent the standard error of the regression line.

This relationship is much better expressed in the clonal population of *Paulownia elongata* x *fortunei* ($F = 90.85$ on 1 and 6 = df, $p = 7,609\text{e-}05$ and adj. $R^2 = 0.9277$) than in *Paulownia tomentosa* ($F = 8.221$ on 1 and 6 = df, $p = 0.02853$ and adj. $R^2 = 0.5078$). This probably was due to the small number of samples in the current analysis. While the height in *Paulownia elongata* x *fortunei* was also proved as a good predictor of the accumulated root biomass ($F = 78.77$, at 1 and 6 = df, $p = 0.0001139$ and adj. $R^2 = 0.9174$), no good linear relationship of root mass to height was found for the clonal population of *Paulownia tomentosa*.

Of the two parameters - base diameter and height - the first one described significantly better the amount of dry stem biomass for both clonal groups (Fig. 12).

This result is in agreement with the already published scientific information about different paulownia clones at juvenile age using nonlinear regression models (Stankova et al.

2016, Stankova et al. 2019). The linear relationship on the base diameter was significantly better expressed in the clonal population of *Paulownia elongata* x *fortunei* ($F = 68.37$ on 1 and 6 = df, $p = 0.000169$ and adj. $R^2 = 0.9059$) than in *Paulownia tomentosa* ($F = 12.39$ on 1 and 6 = df, $p = 0.01252$ and adj. $R^2 = 0.6193$). The total height as a parameter was also a good predictor of the stem biomass of *Paulownia elongata* x *fortunei* ($F = 158$ on 1 and 6 = df, $p = 1.522$ and adj. $R^2 = 0.9573$).

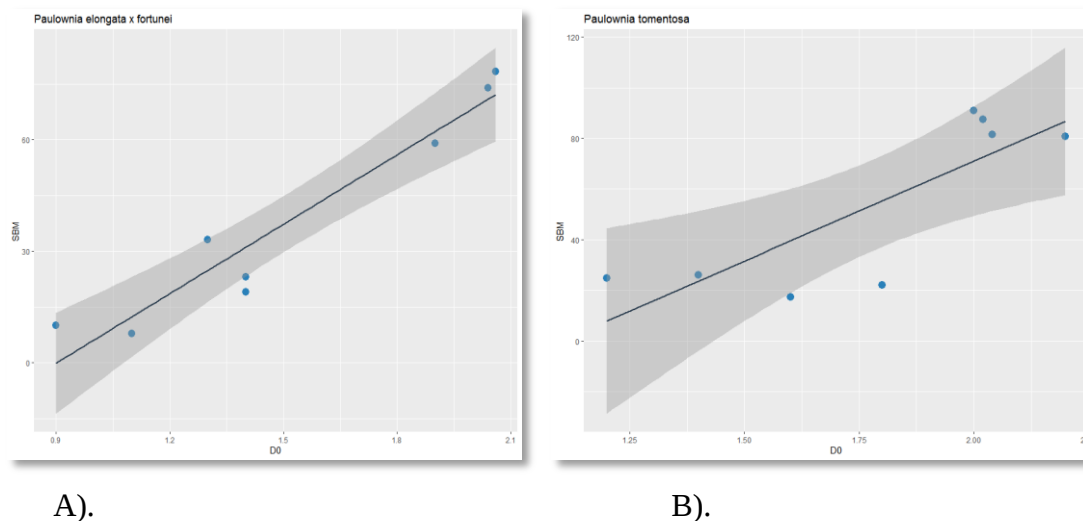


Fig. 12. Linear relationship between base diameter (D_0) and stem biomass (SM) observed for *Paulownia elongata* x *fortunei* (A) and *Paulownia tomentosa* (B). The gray bands around the line represent the standard error of the regression line.

The base diameter was also a better predictor of the total dry biomass produced by both *Paulownia elongata* x *fortunei* and *Paulownia tomentosa* clonal populations. The linear model for *Paulownia elongata* x *fortunei* expressed this relationship significantly better ($F = 65.75$, at 1 and 6 = df, $p = 0.000189$ and adj. $R^2 = 0.9024$) than in *Paulownia tomentosa* ($F = 18.73$, at 1 and 6 = df, $p = 0.004942$ and adj. $R^2 = 0.7169$).

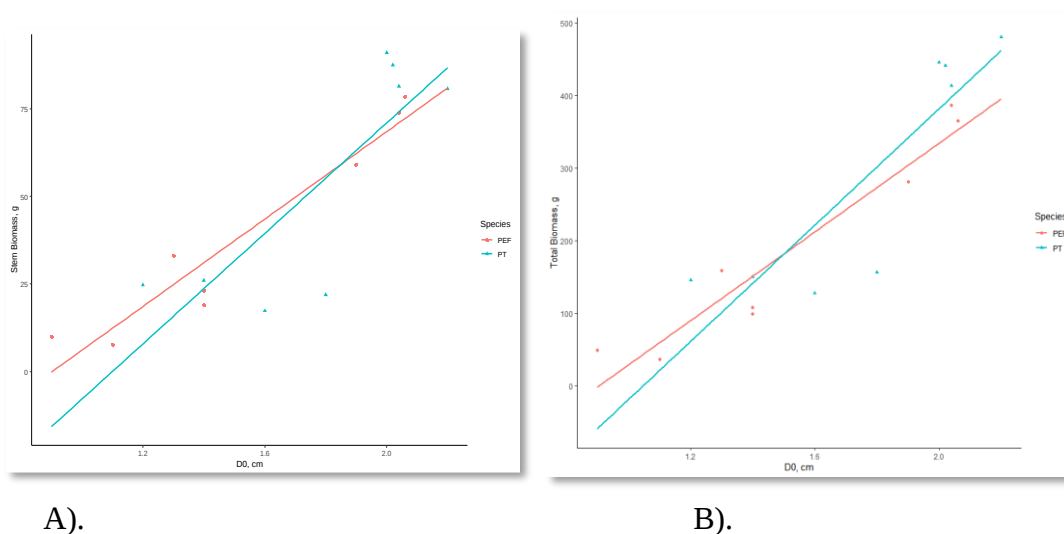


Fig. 13. Response of stem biomass (A) and total biomass (B) to the base diameter D_0 , cm of *Paulownia elongata* x *fortunei* (PEF) and *Paulownia tomentosa* (PT).

Similar to the result above, height as a parameter described better the linear relationship to the total dry biomass produced in the clonal population of *Paulownia elongata* x *fortunei* ($F = 94.16$, at 1 and 6 = df, $p = 6,873e-05$ and adj. $R^2 = 0.9301$). In conclusion, it can be confidently accepted that the base diameter is a good predictor of stem biomass and total dry biomass produced in *Paulownia tomentosa*. This was even more strongly valid for the clonal group of *Paulownia elongata* x *fortunei*, for which the height was also proven as a predictor. All linear models in the clonal population of *Paulownia elongata* x *fortunei* were more accurate than those in *Paulownia tomentosa*. It can be concluded that plants with a base diameter more than 1.5 cm accumulate a larger amount of total dry biomass in *Paulownia tomentosa* at this early stage of their development (Fig. 13).

No linear relationship was found between LAR and RHR and either base diameter or height of the plants. Significant differences between LAR and RSR of *Paulownia elongata* x *fortunei* and those of *Paulownia tomentosa* also were not found. The mean value of the specific leaf area obtained were 137.996 ± 34.515 (cm²/g) and $134,691 \pm 75,428$ (cm²/g) for *Paulownia tomentosa* and *Paulownia elongata* x *fortunei*, respectively. These values are significantly lower than those found for *Populus nigra* at the same age, grown under the same environmental conditions (Gyuleva et al., 2020). The mean values of RSR were 1.58 ± 0.643 and 1.47 ± 0.304 for *Paulownia tomentosa* and *Paulownia elongata* x *fortunei*, respectively.

The lower values of the specific leaf area and LAR are considered indicators of the growth on poorer soils (North et al., 2007). This is not the case of the present study due to the preliminary enrichment of the soil with manure. It can be assumed, however, with high probability, that the climatic conditions and the light in our environment were of the main factors arresting the growth and development of the *Paulownia* species tested (Frey, 2004; Anderegg et al., 2012; Caldwell and O'Hara, 2017). It can be concluded that *Paulownia tomentosa* and *Paulownia elongata* x *fortunei* were cultivated in marginal, to their native areal, zone. As it is known from the literature (Zhu et al. 1986), *Paulownia* species develop a deep root system. The root biomass accumulation, proven here, indicates its function of a reservoir of vital building components (Caldwell and O'Hara, 2017; Landhäusser and Liefers, 2002; Shepperd and Smith, 1993; Zhu et al., 1986) that possesses reliable mechanism to maintain high regenerative capacity after removal of the frozen stems.

Conclusion

During the first stage of this study, height and diameter growth were assessed depending on the growing area and age during the first growing season, the leaf area and the survival of *Paulownia tomentosa* and *Paulownia elongata* x *fortunei* clonal groups during the winter season were evaluated. Undoubtedly, the age factor had stronger influence on growth, than the growing space and respectively explained high percentage of the variation. The growing space as a factor explained a higher percentage of the variation in height and a smaller percentage of the variation in diameter. *Paulownia elongata* x *fortunei* lost a much higher percentage of stems due to frost compared to *Paulownia tomentosa*, but both clonal populations maintained a high regenerative capacity of their root systems during the next growing season. During the second stage of this study, an analysis of the localization of plant biomass to the leaves, stems and roots of the two clonal populations was performed. It was shown that *Paulownia elongata* x *fortunei* clonal population accumulated more stem dry

biomass and *Paulownia tomentosa* clonal population accumulated more leaf mass. Both clonal populations accumulated more than half of the total biomass in their root systems. By using linear relationships between the different functional and metric traits of the plants, it was proven that the base diameter is a good predictor of total biomass, and the leaf area and mass are well predicted through the root biomass for both clonal populations.

Some species peculiarities were also found, such as the larger amount of leaf mass of *Paulownia tomentosa* than *Paulownia elongata* x *fortunei* for the same root weight and the larger leaf area for the same tree height. The results showed that the tested clonal population of *Paulownia elongata* x *fortunei* is not appropriate for bioenergy purposes cultivation in areas with climatic conditions and altitude, identical with the Sofia region. The study outcomes would benefit environmentalists, carbon sequestration of plants, policy-makers and various land users.

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Ameliorative Effect of Biochar Application on Selected Chemical Properties of Acidic Soil

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Abstract

This research was conducted at the screenhouse of Biological Science, Usmanu Danfodiyo University, Sokoto, Nigeria, to determine the ameliorative effect of biochar application on selected chemical properties of acidic soil. The acidic soil used for the experiment was collected from Centre for Agricultural and Pastoral Research (CAPAR), Dabagi, Usmanu Danfodiyo University, Sokoto while the biochar used was obtained from Labana Rice Mill, Brinin-Kebbi, Kebbi State. The biochar was applied at rate of 0, 10, 20, 30, 40, 50, 60 and 70 t/ha. The experiment was laid out in a completely randomized design (CRD) and replicated three times. Data were collected on some soil chemical properties and were subjected to Analysis of Variance (ANOVA) and significant means were separated using Duncan's Multiple Range Test at 5% level of significance. The results showed that successive increment in biochar rates virtually increased all the soil chemical properties tested for, with 70 t/ha of biochar rate significantly ($p \leq 0.05$) higher than the other biochar rates. A field trial of long term experiment with lower doses of biochar rates supplemented with inorganic fertilizer should be conducted in order to have more feasible doses of biochar in the remediation of acidic soil.

Keywords: acidic soil, biochar, chemical properties, remediation, screenhouse

Introduction

Soil acidity is a serious challenge facing agricultural producers in tropical and subtropical regions and limits legume productivity (Bordeleau and Prevost, 1994). The high concentration of H^+ ions causes the solubility of Al, Mn, and Fe, which becomes strong inhibitors of symbiosis (Whelan and Alexander, 1986) and nutrients deficiency. Some soils can be naturally acidic (low pH) while others are much more alkaline. The soil with lower pH

(highly acidic) has detrimental effect on soil chemical properties as well as the growth and yields of the crops that are sensitive to acidic condition. In acidic soils the availability of the major plant nutrients such as nitrogen, phosphorous, potassium, sulfur, calcium, magnesium, organic carbon among others is reduced and may be insoluble (Gazey and Davies, 2009). Hence, soil acidity could lead to the deterioration of the soil and nutrient deficiency among others (Owolabi et al., 2003).

Several soil liming and amendment materials were widely used to increase soil pH including dolomite, calcite, and lime and which in turn reduce exchangeable aluminium, iron, and hydronium in the soil. The above mentioned acidic soil remediating materials are relatively expensive to afford by the subsistence farmers. However, there has been increased interest on alternative liming agents with multiple benefits such as pyrolytic biochars which can be used to improve soil fertility and to store carbon (C) in the soil (Steiner et al., 2007; Nguyen & Lehmann, 2009; Yuan & Xu, 2011). Biochar is thermally-decomposed (pyrolysis) in a low or no oxygen environment (Gaskin et al., 2008) and deliberately applied to the soil to improve its properties (Lehmann and Joseph, 2009) and soil pH (Xu et al., 2006). Sohi et al., (2010) reported an increase in sorption capacity for nutrients by acidic soil due to application of biochar while Van Zwieten et al. (2009) reported a reduction in the exchangeable acidity as a result of biochar application.

However, information on the effect of biochar on the chemical properties of acidic soil in the study area is not adequate and therefore there is the need to check if biochar could be used to raise the soil pH as well as improving its chemical properties. This research aims to evaluate the ameliorative effect of biochar on some chemical properties of acidic soil of Dabagi, Sokoto State.

Materials and Methods

Study area

The research was conducted at the Screenhouse of the Department of Biological Science, Usmanu Danfodiyo University, Sokoto, Nigeria. Sokoto State is located in North-western part of Nigeria, near the confluence of the Sokoto River and Rima River, situated between latitudes 11° 13' to 13° 50' N and longitudes 4° and 6° E having altitude of 350m above sea level (Sokoto State Tourist Guide, 2010). The climate of Sokoto State is hot, semi-arid, with long dry season characterized by cool dry air during harmattan around November to February and hot dry air during hot season from March to May, with short rainy season ranging from June to October with average of 630 mm, relative humidity range from 26-29%. Minimum temperature ranges between 19° C and 27° C and maximum temperature ranges from 30 - 40° C and wind direction generally North-westerly in the dry season and South-westerly in the wet season and wind speed range between 1.7 and 4.0 m/s (Jibrillah et al., 2018).

Source of biochar

The biochar was collected from Labana Rice Mill, Birnin-Kebbi, Kebbi State. The biochar was produced using rice husk as the feedstock by pyrolysis for 40-60 minutes at temperature range of about 250°C–300°C. Biochar was crushed and passed through a 2 mm sieve before chemical characterization. The nutrients composition of biochar analysis

includes; pH, organic carbon, available P, total N, cation exchangeable capacity (CEC) and exchangeable bases (Mg, K, Na and Ca).

Experimental design and layout

The biochar was incorporated at the rates of 0, 10, 20, 30, 40, 50, 60 and 70 t/ha which is equivalent to 0, 25, 50, 75, 100, 125, 150 and 175g of biochar in to 5kg of acidic top soil collected from Centre for Agricultural and Pastoral Research (CAPAR), Dabagi Area, Sokoto State at a depth of 0-15 cm. SAMNUT-24 was used as test crop and the experiment was laid out in a Completely Randomized Design (CRD) and replicated three times in the screenhouse.

Soil sampling and laboratory analysis

The soil was sampled before the experiment and also from each bucket in the screenhouse after harvest and chemical characteristics of the soil were determined using standard methods. The soil pH was determined using glass electrode pH meter (McLean, 1971). Soil organic carbon was determined using oxidation method of Nelson and Sommers (1982). Total nitrogen was determined by Micro Kjeldahl digestion method (Bremmer and Mulvaney, 1982). Available phosphorus was determined using bray-1 method (Bray and Kurtz, 1945). Calcium (Ca) and magnesium (Mg) were determined using EDTA titration method while potassium (K) and sodium (Na), in the extract were read using flame photometer. The cation exchange capacity of the soil samples was determined using normal neutral ammonium acetate (Davis and Freitas, 1970).

Data analysis

The data obtained was subjected to Analysis of Variance (ANOVA) and the significance differences among means were separated using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Results and Discursion

Chemical properties of soil and biochar used for the study

The pre-sowing analyses of the soil and biochar used for this experiment were presented in Table 1. The soil pH was 5.02 and was rated as strongly acidic while that of biochar was 8.14 and was rated as moderately alkaline (Soil survey division staff, 2017). The available phosphorus of the soil was 1.14 mg/kg while that of biochar was 1.53 mg/kg and both were rated as very low according to Landon (1984). The percentage organic carbon of the soil was 0.42% and was rated as low while that of biochar was 7.78% and was rated as medium (Landon, 1984). The total nitrogen of the soil was 0.025% and is rated as low while that of biochar was 0.280% and was rated as medium (Enwezor et al., 1989). The cation exchange capacity (CEC) of the soil was 1.92 cmol/kg and was rated as low while that of biochar was 10.16 cmol/kg and was rated as medium (Esu, 1991).

The exchangeable bases of the soil viz.; Ca was 0.55 cmol/kg and was rated as low (0-2 cmol/kg), Mg was 0.50 cmol/kg and was rated as medium (0.3-1.0ccmol/kg), K was 0.10 cmol/kg and was rated as low (0-0.15 cmol/kg) and Na was 0.17 cmol/kg and was rated as medium (0.1-0.3 cmol/kg) (Enwezor et al., 1989). Similarly, the exchangeable bases of the biochar viz.; Ca was 0.50 cmol/kg and was rated as low (0-2 cmol/kg), Mg was 0.85 cmol/kg and was rated as medium (0.3-1.0 cmol/kg), K was 5.83 cmol/kg and was rated as high (>0.3

cmol/kg) and Na was 2.18 cmol/kg and was rated as high (>0.3 cmol/kg) (Enwezor et al., 1989).

Table 1. Chemical properties of soil and biochar used for the study

Properties	Biochar	Soil
pH	8.14	5.2
Organic Carbon (%)	7.78	0.42
Phosphorus (mg/kg)	1.53	1.14
Total Nitrogen (%)	0.28	0.025
CEC (cmol/kg)	10.16	1.92
Exchangeable bases (cmol/kg)		
Magnesium	0.85	0.5
Calcium	0.5	0.55
Sodium	0.18	0.17
Potassium	5.83	0.1

Effect of biochar on soil pH, organic carbon, total nitrogen and available phosphorus (P) at 9 and 15 WAA (weeks after amendment with biochar).

The soil pH after biochar amendment in the soils was presented in Table 2. At 9 weeks after amendment with biochar (WAA) the biochar rates of 50, 60 and 70 t/ha recorded significantly ($p \leq 0.05$) higher values of soil pH when compare to 0, 20 and 30 t/ha of biochar rates. However, 50, 60 and 70 t/ha were statistically comparable with 40 t/ha at 9 WAA. Similarly, at 15 WAA the biochar rates of 50, 60 and 70 t/ha were also significantly ($p \leq 0.05$) higher when compare to 0, 10 and 20 t/ha but statistically comparable with 30 and 40 t/ha. The soil pH increased with increasing quantity of biochar and this could be as a result of alkaline nature of rice husk biochar. Similar result was found in the work done by Glaser *et al.* (2002); Lehmann and Rondon (2006), who reported that biochar, can serve as a liming agent, resulting in increased pH of the soil.

Significant differences were observed among biochar application rates on available P where 60 and 70 t/ha biochar rates recorded significantly ($p \leq 0.05$) higher available P than 0, 10 and 20 t/ha, but statistically similar to 30, 40, and 50t/ha of biochar rates at 9 WAA. Similarly, at 15 WAA all the biochar rates were significantly ($p \leq 0.05$) higher in available P than control (0 t/ha). This might be attributed to the ability of biochar pH to break the complexes of Al and Fe in soil and thereby releasing more phosphorus. This is in conformity with the research of Prasad (1992) who reported that soil available P increased significantly under liming due to lowering P fixation by other elements (Al, Mn, and Fe). Sood and Bhardwaj (1992) and Rahman et al., (2001) have also reported that soil available P was higher under limed over the none-limed plots.

Similarly, the percentage organic carbon increased with increasing amount of biochar rates with 70 t/ha significantly ($p \leq 0.05$) higher in organic carbon content when compared to other biochar rates though comparable with 60 t/ha at 9 WAA. But at 15 WAA, the biochar rates of 40, 50, 60 and 70 t/ha were significantly ($p \leq 0.05$) higher than 0, 10 and 20t/ha rate of biochar application but statistically similar with 30 t/ha.

The increase observed in the OC could be as a result of the organic carbon content in the biochar which is capable of increasing the soil organic carbon after amendment. Similar result was reported by Nigussie et al., (2012); Lehmann (2007); Zhang et al., (2012).

At 9 WAA, the total nitrogen followed the same trend as that of organic carbon with 70 t/ha significantly ($p \leq 0.05$) higher than other biochar rates though statistically similar to 60 t/ha of biochar rates. At 15 WAA, the biochar rates 60 and 70 t/ha were significantly ($p \leq 0.05$) higher than other biochar rates of application but statistically the same with 50 t/ha. Liang *et al.* (2006) stated in his research that the total nitrogen also increased with the successive increment in the rate of biochar and this could be due to decomposition which might have occurred when biochar is added to soil, which could induce net immobilization of inorganic N already present in the soil solution.

Effect of biochar on exchangeable bases and cation exchange capacity (CEC) at 9 and 15 WAA (Weeks After Amendment with biochar)

Table 3 indicated that addition of biochar increased all the exchangeable bases (Ca, Mg, Na and K) and cation exchange capacity of the soil at each successive increase in the rate of biochar application. At 9 WAA, no significant difference was observed among the biochar application rates in calcium (Ca) which could be as result of slow mineralization of biochar. But at 15 WAA, the biochar rates of 60 and 70 t/ha recorded significantly ($p \leq 0.05$) higher in calcium than other biochar application rates. 70 t/ha of biochar rate recorded significantly ($p \leq 0.05$) higher values of Mg when compared to other biochar rates and statistically comparable with 20, 30, 40, 50 and 60 t/ha at 9 WAA. But at 15 WAA, the biochar rates of 40, 50, and 70 t/ha were significantly ($p \leq 0.05$) higher than other biochar rates but statistically comparable with 30 and 60 t/ha.

There were significant differences among biochar rates in Na at 9 WAA, where 60 and 70 t/ha recorded significantly ($p \leq 0.05$) higher values of Na than other biochar rates. At 15 WAA, the biochar rate of 70 t/ha was significantly ($p \leq 0.05$) higher than 0, 10, and 20, and 30 t/ha though statistically comparable with 40, 50 and 60 t/ha. Potassium increased with increasing rate of biochar with 70 t/ha producing significantly ($p \leq 0.05$) higher than 0, 10 and 20 t/ha though comparable with 30, 40, 50 and 60 t/ha at 9 WAA. However, at 15 WAA there were no significant differences among the biochar rates in the amount of potassium.

Table 2. Soil pH, organic carbon, total nitrogen and available phosphorus (P) at 9 and 15 WAA.

Biochar rates (t/ha)	Soil pH		AP		OC %		TN	
	9	15	WAA	15	WAA	15	WAA	15
0	5.01 ^c	5.06 ^d	1.10 ^c	1.40 ^b	1.32 ^c	1.07 ^c	0.033 ^f	0.036 ^d
10	5.61 ^{bc}	5.73 ^c	1.40 ^b	1.98 ^a	1.34 ^c	1.26 ^c	0.035 ^{ef}	0.040 ^{cd}
20	5.70 ^b	6.06 ^{bc}	1.58 ^b	2.00 ^a	1.35 ^c	1.33 ^{bc}	0.037 ^{de}	0.043 ^{cd}
30	5.82 ^b	6.25 ^{abc}	1.63 ^{ab}	2.04 ^a	1.40 ^c	1.62 ^{ab}	0.041 ^{cd}	0.044 ^{cd}
40	5.95 ^{ab}	6.57 ^{ab}	1.66 ^{ab}	2.07 ^a	1.42 ^c	1.65 ^a	0.041 ^{cd}	0.048 ^{bc}
50	6.49 ^a	6.68 ^a	1.74 ^{ab}	2.11 ^a	1.52 ^{bc}	1.66 ^a	0.044 ^{bc}	0.055 ^{ab}
60	6.66 ^a	6.76 ^a	2.07 ^a	2.21 ^a	1.76 ^{ab}	1.82 ^a	0.046 ^{ab}	0.058 ^a
70	6.62 ^a	6.83 ^a	2.07 ^a	2.22 ^a	1.93 ^a	1.93 ^a	0.049 ^a	0.062 ^a
SE ±	0.13	0.13	0.05	0.06	0.08	0.06	0.001	0.002
LS (p≤0.05)	*	*	*	*	*	*	*	*

OC = Organic carbon, TN = Total Nitrogen, AP = Available Phosphorous, Means within the same column with the same letters are not significantly different according to Duncan Multiple Range Test at (p≤0.05), SE = Standard error, * = Significant at (p≤0.05), WAA = Weeks After Amendment with biochar.

Table 3. Effect of biochar on Ca, Mg, Na, K and CEC at 9 and 15 WAA

Biochar rate (t/ha)	Ca		Mg		Na		K		CEC	
	9	15	9	15	9	15	9	15	9	15
0	0.58	1.16 ^d	2.17 ^c	0.53 ^c	0.20 ^c	0.17 ^c	0.24 ^c	0.11	1.36 ^d	0.85 ^d
10	0.63	1.66 ^{cd}	3.00 ^{bc}	0.73 ^c	0.41 ^{bc}	0.19 ^c	0.30 ^{bc}	0.15	2.18 ^c	1.77 ^{cd}
20	0.90	2.23 ^{cd}	3.40 ^{abc}	1.07 ^{bc}	0.45 ^{bc}	0.20 ^c	0.31 ^{bc}	0.19	2.37 ^c	2.30 ^{cd}
30	0.98	2.37 ^{bcd}	3.50 ^{abc}	1.64 ^{ab}	0.51 ^b	0.26 ^{bc}	0.48 ^{abc}	0.21	3.07 ^c	2.90 ^{cd}
40	1.03	2.93 ^{bc}	3.83 ^{ab}	2.18 ^a	0.60 ^b	0.32 ^{abc}	0.47 ^{abc}	0.31	3.15 ^{ab}	3.19 ^{bc}
50	1.22	3.13 ^b	4.03 ^{ab}	2.05 ^a	0.93 ^b	0.45 ^{abc}	0.44 ^{abc}	0.41	3.58 ^{ab}	3.40 ^{bc}
60	1.40	4.80 ^a	4.13 ^{ab}	2.17 ^{ab}	0.94 ^a	0.52 ^{ab}	0.62 ^{ab}	1.10	3.73 ^{ab}	4.92 ^{ab}
70	1.72	5.23 ^a	4.67 ^a	2.40 ^a	0.94 ^a	0.60 ^a	0.68 ^a	1.15	3.82 ^a	6.68 ^a
SE ±	0.17	0.31	0.21	0.16	0.06	0.04	0.04	0.13	0.18	0.41
LS (p≤0.05)	Ns	*	*	*	*	*	*	ns	*	*

Ca = Calcium, Mg = Magnesium, Na = Sodium, K = Potassium and CEC = Cation Exchange Capacity. Means within the same column with the same letters are not significantly different according to Duncan Multiple Range Test at ($p > 0.05$), SE = Standard error, ns = Not significant, * = Significant at ($p \leq 0.05$) and WAA = Week After Amendment with biochar.

CEC followed the same pattern like other exchangeable bases by having 70 t/ha significantly higher than other application rates of biochar. At 9 WAA, the biochar rate of 70 t/ha was significantly ($p \leq 0.05$) higher in CEC than other biochar application rates but statistically similar to 40, 50 and 60 t/ha. Likewise at 15 WAA, 70 t/ha was rated to be significantly ($p \leq 0.05$) higher in CEC than other biochar rates but statistically the same with 60 t/ha.

Generally, the research indicated that biochar application to acidic soil increases the CEC and exchangeable bases, thereby improving the fertility of the soil and invariably increases the soil productivity. This result is in line with the previous researches by Yusif et al. (2016) who reported that biochar application have the potential to improve soil chemical properties. Rondon et al. (2007), Major et al. (2010), Liang et al. (2006), Agegnehu et al. (2016), Hardy et al. (2017) and Cheng et al. (2006) reported increased in CEC of soil amended with biochar.

Conclusion

The high rates of biochar have the potential to improve the soil chemical properties in acidic soil but may increase the amount of Na which is toxic to plant at a given quantity and can lead to sodicity of soil.

Recommendations

The biochar application rates of 50 t/ha was comparable with 60, and 70 t/ha in most cases and therefore be recommended for improvement of soil chemical properties of acidic soil. Biochar with low concentration of Na should be used to avoid toxicity and sodicity of soil. A field trial of long term experiment with lower doses of biochar rates supplemented with inorganic fertilizer should be conducted in order to have more feasible doses of biochar in the remediation of acidic soil.

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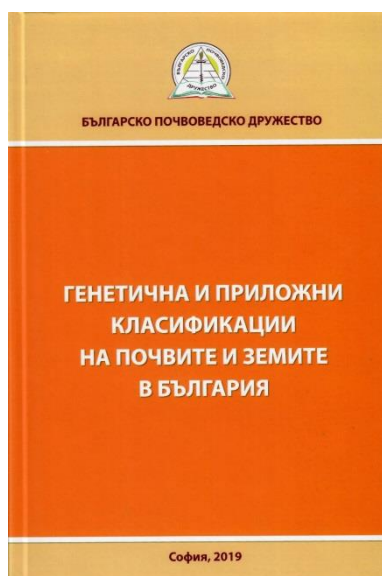
New Book

“Genetic and Applied Classifications of Soils and Lands in Bulgaria”

Under the general editorship of Prof. Dr. Metodi Teoharov

Bulgarian Soil Science Society - 2019

ISBN 978-619-90414-3-7



This scientific book was developed by decision of the executive board of the Bulgarian Soil Science Society. For development of this issue were involved the best scientists and students from ISSAPP "Nikola Poushkarov", Agricultural University - Plovdiv and University of Forestry-Sofia. This book is based on modern rules, criteria and diagnostics, FAO, European and world standards. The classifications were prepared and discussed at a scientific session held on November 14, 2018 with the scientific community of ISSAPP "N. Poushkarov" and other scientific and academic organizations. The book can be used in the curricula of university and secondary schools with agronomic, forestry, agricultural, economic, biological and environmental characteristics and in the development and use of various scientific, scientific-applied and technological projects at national and international level. The book will be useful for

agricultural, forestry and conservation organizations to modernize projects and develop projects for management of agricultural, forest, unprotected and protected areas, for projects and EIA abroad for ecology and use of the environment.

This book is an important investment in the future for the development of science and practice.

The book was published under the general editorship of Prof. Dr. Metodi Teoharov. Bulgarian Soil Science Society, 2019

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Election of Honorary Members of IUSS

The Executive Board of the Bulgarian Soil Science Society congratulates prof. Dr Rainer Horn for the prize on being awarded for Honorary Member of IUSS.

The IUSS Inter-Congress Meeting took place virtually (instead of a physical meeting in Glasgow, Scotland, which had been planned for September 2020) on 18-23 November 2020. It comprised three sessions each of the Executive Committee Meeting and the IUSS Council Meeting as well as one Research Forum Meeting.

Main topics were the past and future activities of IUSS Presidents, Secretariat and Treasurer, Standing Committees, Divisions, Commissions and Working Groups as well as the Research Forum, internal and international matters of the IUSS, preparation of the 22nd World Congress of Soil Science (WCSS 2022 in Glasgow, Scotland), outlook to the Centennial in 2024 as well as the presentations of societies intending to bid for the WCSS in 2030.

Furthermore the election of the new Honorary members was carried out. The following scientist who provided distinctive contributions to soil science in general and the IUSS in particular were elected:

Rainer Horn - Germany
Jozef A. (Seppe) Deckers - Belgium
Flavio Anastacio de Oliveira Camargo - Brazil
Carmelo Dazzi - Italy
Kazuyuki Inubushi - Japan
Kye-Hoon 'John' Kim - Korea
Bal Ram Singh - Norway
Pavel Krasilnikov - Russia
Rosa M. Poch Claret - Spain
Alfred Hartemink - USA

Congratulations to these candidates on being awarded Honorary Membership of the International Union of Soil Sciences!

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Bulgarian Journal of Soil Science publishes four categories of papers and the authors must indicate the category of their submission in the cover letter:

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- Review papers (invited or consulted with the Editor prior to preparation of the manuscript) with maximum length of 25-30 pages (according to the importance of the material).
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Journal articles

- Atanassova, I., S. Doerr, 2010. Organic compounds of different extractability in total solvent extracts from soils of contrasting water repellency. *European Journal of Soil Science*, 61, 298-313.
- Calderón, H. W., 1982. The content and composition of humus in arid alluvial soil of Peru. *Soil Science, Academy of Sciences of USSR, Moscow*, 8, 53-59. (in Russian).

Books

- Sparks, D. L., 2002. *Environmental Soil Chemistry*, 2nd Edition, Academic Press, San Diego, CA.

Book chapters or conference proceedings

Petrova, R., 1989. Loss of Humus Soil in the Process of Reclamation of Damaged Terrains. In: N. Sherbanova, R. Nacheva (Eds.). *Proceedings from Fourth National Conference on Soil Science 'Problems of Soil Science in Intensive Agriculture'*. BSSS, N. Poushkarov Soil Science and Yield Programming Institute, Sofia, 203-206, (In Bulgarian).

Online publications

Atanassova, I., S. H. Doerr, 2011. Changes in Soil Organic Compound Composition Associated with Heat-Induced Increases in Soil Water Repellency. *European J. of Soil Science*, DOI: <http://10.1111/j.1365-2389.2011.01350.x>

Dissertations and Philosophy Doctor Theses

Hristova, M., 2013. content and availability of microelements-metals in Technogenic soils. Ph.D. Thesis. Agricultural Academy, ISSAPP 'N. Poushkarov', Sofia, 140. (In Bulgarian)

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